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CONTENTS

Review Article
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FRONTIERS IN BIOTECHNOLOGICAL INTERVENTIONS FOR RICE IMPROVEMENT

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ABSTRACT

Rice is the world's most important cereal food crop and a staple food for more than half of the world's population. The increase in food demand along with decline in the availability of arable land and water resources will be indeed difficult to meet the challenges only with the application of conventional plant breeding techniques and tools. Biotechnological tools as a viable solution to meet the rice production and productivity and helps to sustain the food security. The molecular techniques i.e., genomics-assisted selection, genetic engineering, functional genomics, genome editing and genome wide association studies has opened up novel opportunities for enhancing rice yields beyond the limits imposed by conventional breeding. These broad applications of biotechnology expected to contribute both directly and indirectly towards rice improvement. This review focuses on possibilities for the application of biotechnology tools in the genetic improvement of rice breeding.

Rice (*Oryza sativa* L.) is the major food crop of the world, global consumption of rice is steadily increasing from 440 million tonnes (2011) to 480 million tons (2017) and expected to increase about 650 million tons by 2050 (www.statista.com/statistics; Wijerathna, 2015). In the last six decades, rice yield growth has reached a plateau and no significant increase is being realized in productivity levels due to various biotic, abiotic stresses and several environmental factors. To cope up with the challenge of increasing the crop production, harnessing modern tools of biotechnology are essential to meet the demand, since these tools can create the novel variability available in germplasm as well across the species.

Tremendous advancements in biotechnology, enabled us to use various tools which encompasses use of different types of molecular markers in plant breeding as marker-assisted selection, genomics-assisted selection, marker-based cloning, Genome wide association studies (GWAS), allele mining which hasten the advancement of cultivars possessing trait of interest in lowest number of generations. At the same time progress in genomics and plant genetic engineering led to the availability of latest tools like activation tagging, genomic assisted mutagenesis, RNA interference, Genome editing empowers the developers to use the native/foreign genes either to express or inhibit the function of the gene to improve the different traits. Overall, this review present various types of molecular tools are being used or developed for the improvement of rice production with enhanced quality.

Rice improvement is being done through the conventional plant breeding techniques using donors from the germplasm from long time. The speed in the development of cultivars increased after the availability of various types of markers from characterized genes, expressed sequence tags (ESTs) and genome sequencing. Among the more important and popular molecular markers are simple sequence repeats (SSRs), single-nucleotide polymorphisms (SNPs) and functional markers (markers developed from gene sequence). Availability of gene sequence variations from some *Oryza* species is also a great significance for molecular breeding. Increasingly, markers are being applied to the selection of loci controlling traits that are difficult to select phenotypically. A perfect marker allows breeders to track specific alleles within pedigrees and populations and to minimize linkage drag flanking the gene of interest.

Marker-assisted selection (MAS)

Marker-assisted selection (MAS) refers to the use of DNA markers that are tightly-linked to target loci as a substitute to assist phenotypic screening

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and thereby helps in tracking the inheritance of the particular trait (Ribaut and Hoisington, 1998). The basic assumption is that the DNA markers can reliably predict phenotype and this depends on the linkage distance between the marker(s) and the gene/QTL of interest controlling the trait phenotype. Markers should be deployed only for those traits, which cannot be easily scored for phenotype or those governed by recessive genes. Generally, very tightly linked markers with close linkage (< 2 cM) with the gene of interest or markers flanking the gene (< 5 cM on either side of the gene) should be used in current breeding programs (Jena and Mackill, 2008). The individual plants having particular marker allele can be selected without exposing the plants to the selection pressure as the presence of marker indicates the presence of the desired characteristics in the particular plant. As on date, SSR/SNP markers are considered the most ideal markers for MAS in rice. SNPs have become markers of choice in rice breeding as they are highly abundant and amenable for high throughput genotyping. Some of the success stories related to application of MAS in rice are enlisted in Table 1. There are three major MAS schemes presently adopted in crop breeding i.e., Marker-assisted backcross breeding (MABB), Marker-assisted recurrent selection (MARS) and Marker-assisted genome wide selection.

The Marker- assisted backcross breeding (MABB) involves backcross breeding in which the favorable allele(s) of gene(s) of interest are specifically transferred from a donor to the recurrent parent through 2-3 cycles of backcrossing. Plants possessing the target allele(s) for the gene(s) of interest are selected with the help of gene-specific molecular marker(s), instead of subjecting to phenotypic selection. Generally, backcross breeding programs involve transfer of a limited number ($n \leq 5$) of genes from donor to the recurrent parent (Neeraja *et al.* 2009). With conventional backcrossing, it takes a minimum of five to six generations to recover the recurrent parent genome. However, several studies clearly demonstrated that 2-3 backcross generations were sufficient to recover the recurrent parent genome (Joseph *et al.* 2004; Balachiranjeevi *et al.* 2015; Ellur *et al.* 2016a, 2016b). MABB approach has limited applicability, when the variation is controlled by minor QTLs. To overcome these limitations, **marker-assisted recurrent selection** (MARS) approach has been advocated. In this approach, once gene(s)/QTL(s) controlling traits are discovered, markers are used as predictors of the breeding values to increase the frequencies of favourable alleles at these loci. This strategy is most effective when the gene(s)/QTLs involved have major effects and prior information is available on marker-trait associations/linkage and when the number of loci selected are more ($n \geq 20$).

Unlike MARS, in genome wide selection, any prior information on marker-trait associations is not required and it can be used to select multiple loci of small genetic effects. In this approach, populations are extensively genotyped to give full genome coverage and phenotyped for individual lines in the test populations. Subsequently, these data allow the prediction of phenotypic performance of an individual on the basis of whole-genome marker surveys. This approach will be highly suitable for combining several small effect QTLs for different agronomical traits.

Applications

Marker-assisted breeding (MAB) has the following distinct advantages as compared to conventional phenotypic-selection based breeding (Collard and Mackill, 2008). MAB facilitates cost effective, time saving and non-destructive assay(s). Selection can be carried out at any growth stage from seed to maturity, facilitates differentiation of homozygous segregants from heterozygous ones in backcross, bulk and pedigree breeding methods and thus facilitating early generation selection of superior recombinants, particularly for those traits controlled by recessively inherited genes. Screening can be done even without having the incidence of pests/disease. The progress and prospects of molecular mapping and marker-assisted selection related to different agronomically important traits are detailed below.

Marker-assisted breeding for biotic stress resistance in rice

Three diseases (viz., bacterial blight, blast and rice tungro disease) and two pests (gall midge and BPH) of rice can be managed through host-plant resistance by deploying molecular markers linked to the resistance genes. The progress of research related to MAS for resistance breeding of biotic stresses are given in the table 1.

Table 1: Present status of MAS work for combating biotic stresses

S.No	Trait	Genes transferred	Background	Reference
1.	Blast	*Pi1+ Piz-5 + Pita*	Co39	Hittalmani *et al.* (2000)
		Pi-d(t)1+ Pi-b + Pita2	G46B	Chen *et al.* (2004)
		Pish + Pib	Co39	Koide *et al.* (2010)
		Multiple blast resistance QTLs	IR64	Sreewongchai *et al.* (2010)
		Pi1 + Piz5	PRR78	Gouda *et al.* (2012)
		Pi1+ Pi2 + D12	Jin28B	Jiang *et al.* (2012)
		Pi1 + Pi2	Rongfeng B	Fu *et al.* (2012)
		Piz + Pi5	Carnaroli and Baldo.	Urso *et al.* (2013)
		pi21+ Pi34 + Pi35	Koshihikari	Yasuda *et al.* (2014)
		Pi2	GZ63-4S	Jiang *et al.* (2015)
		Pi54	Improved Samba Mahsuri	Madhavi *et al.* (2012)
		Pi2 + Pi54	Improved Samba Mahsuri	Madhavi *et al.* (2016)
		Pi2 + Pi54	DRR17B	Balachiranjeevi *et al.* (2015)
		Pi40	Osmancik97, Halilbey	Beser *et al.* (2016)
		Pi-7(t), Pi-d(t)1, Pir2-3(t), qLN2	MR263	Hasan *et al.* (2016)
		Pi9	GZ63S	Ni *et al.* (2015)
		Pi1, Pi2, Pi33	Kuboyar	Usatov *et al.* (2016)
		Pi54	RPHR1005R	Abhilash *et al.* (2016)
		Pi2 + Pi54	Akshyadhan	Bhaskar *et al.* (2015)
		Pi54	MTU1010	Arunakumari *et al.* (2016)
		Pi1 + Pi54	Tellahamsa	Jamal-Oddin *et al.* (2015)
		Pi-b + Pi-kh	MR219	Tanweer *et al.* (2015a)
		Piz, *Pi2 + Pi9*	MR219	Miah *et al.* (2016)
		Pi54	JGL-1798	Swathi *et al.* (Unpublished)
		Pi1+ Pi2 + Pi54	Swarna-Sub1	Parashuram *et al.* (2014)
		Pi1, Pi2, Pi54, Pizt, Pi9 and *Pi40*	Development of Mono and digenic lines of Samba Mahsuri	Madhav et al (unpublished)
		Pi54	Pusa6B	Singh *et al.* (2012)
		Piz-5 + Pi54	PRR78	Singh *et al.* (2013)
		Pi2 + Pi54	Pusa Basmati1121	Ellur *et al.* (2016b)
2.	Blast and Bacterial blight	*Pi54 + Xa21*	IR58025B	Hari *et al.* (2013)
		Xa21 + Pi54	APMS6B	Srinivasarao, (2010)
		Xa21 + Xa33+Pi2 +Pi54	RPHR1005R	Abhilash *et al.* (2016)
		Pi54 and *Xa21 + Xa13*	MTU1010	Arunakumari *et al.* 2016
		Pi2 + Pi54 and *Xa21 + Xa13*	Samba Mahsuri	Madhavi *et al.* 2016

S.No	Trait	Genes transferred	Background	Reference
		Pi2 + *Pi54* and *Xa21* + *Xa13*	Pusa Basmati6 and Pusa Basmati1121	Ellur *et al.* 2016a
		Pi54 and *Xa21*	IR58025B	Hari *et al.* 2013
		Pi25 and *Xa21+Xa13+Xa5*	R8012	Zhanj *et al.* 2012
		Pi1 + *Pi2* and *Xa23*	RongfengB	Fu *et al.* (2012)
3.	Bacterial Blight	*Xa4+ xa5* + *Xa10*	*Japonica* variety	Yoshimura *et al.* (1995)
		Xa21+ xa13 + *xa5*	New-plant type rice lines	Sanchez *et al.* (2000)
		Xa21+ xa13 + *xa5*	PR106	Singh *et al.* (2001)
		Xa21+ xa13 + *xa5*	IR64	Davierwala *et al.* (2001)
		Xa21 + *xa13*	Pusa Basmati-1	Joseph *et al.* (2004)
		Xa21+ xa13 + *xa5*	Samba Mahsuri	Sundaram *et al.* (2008)
		Xa21	Minghui 63	Chen *et al.* (2000)
		Xa21	R8006 and R1176	Cao *et al.* (2003)
		Xa21+ Xa7	Minghui 63	Zhang *et al.* (2006)
		Xa4+ Xa7 + *Xa21*	*TGMS1*	Perez *et al.* (2008)
		xa13 + *Xa21*	Pusa 6B and PRR68	Basavaraj *et al.* (2010)
		Xa21	KMR3R	Hari *et al.* (2011)
		Xa21+ xa13+ Xa38	Pusa Basmati1121	Ellur *et al.* (2016a)
		xa5+ xa13+ Xa21	Jalmagna	Pradhan *et al.* (2015)
		Xa4+ xa5+xa13+ Xa21	Lalat, Tapaswini	Dokku *et al.* (2013)
		Xa21+xa13	Taraori Basmati, 386 Basmati	Pandey *et al.* (2012)
		Xa4+xa5+ Xa13+ Xa21	Mahsuri	Guvvala *et al.* (2013)
		Xa4+ xa5+ Xa21	Mangeubyeo	Suh *et al.* (2013)
		Xa33	Samba Mahsuri	Natarajkumar *et al.*(2012)
		Xa21	KDML105	Win *et al.* (2012)
		Xa7+Xa21+Xa22+Xa23	Huahui1035	Huang *et al.* (2017)
4.	Rice tungro disease	*qRTV-7*	IR64, MTU1010, CR 1009 and BPT 5204	Sundaram *et al.* (2014)
5.	Gall midge	*Gm2* + *Gm6*	Duokang #1 and Phalguna	Katiyar *et al.* (2001)
6.	Gall midge and Bacterial Blight	*Xa21+xa13+ Gm8*	Improved Samba Mahsuri	Sama *et al.* (2012)
7.	Brown Plant hopper	*Bph1* + *Bph2*	*Japonica* line	Sharma *et al.* (2004)
		Bph18	Junambyeo	Jena *et al.* (2006)
		Bph14 + *Bph15*	Restorer lines (9311 and 1826)	Li *et al.* (2006)
		Bph25 + *Bph26*	T65	Myint *et al.* (2012)

S.No	Trait	Genes transferred	Background	Reference
8.	Combination of biotic stress	*Pi2 + Xa21+Xa33 + Rf3+Rf4*	RPHR1005R	Abhilash *et al.* 2016
		Pi2+Xa21+Xa33+Rf3+Rf4	DRR17B	Balachiranjeevi *et al.* (2015)
		Pi40 + Xa21+Xa5+Xa4+ Bph18	Jinbubyeo	Suh *et al.* (2011)
		Pi9 and *badh2* and *Xa4+ Xa21+Xa27*	Wanhui725	Luo *et al.* 2016

Introgression of QTLs other agronomically important traits

Even though the progress related to molecular mapping and marker-assisted breeding of traits controlled by single genes (for example, bacterial blight resistance, blast resistance etc.) has been tremendous in rice, progress related to molecular mapping and marker-assisted selection of traits controlled by multiple genes/QTLs (for e.g. yield, abiotic stress tolerance, grain quality etc.) has been very limited. Progress in the introgression of QTLs for various traits is given in table -2.

Table 2: Status of QTLs identification and introgression of QTLs for various traits

S.No	Trait	QTLs/gene	Reference
1.	Drought under yield	*qDTY1.1, qDTY2.2, qDTY3.1, qDTY3.2, qDTY6.1, qDTY12.1*	Kumar *et al.* (2014)
2.	Submergence tolerance	*Sub1A*	Xu *et al.* (2006)
		Sub1A in the genetic back ground of Swarna	Neeraja *et al.* (2007)
		Sub1A in the genetic back ground of Samba Mahsuri, IR64 and other popular varieties of Philippines and Indonesia	Septiningsih *et al.* (2009)
3.	Salinity	*Saltol, Skc*	Thomson *et al.* (2010)
4.	Chilling tolerance	*COLD1*	Yun *et al.* (2015)
5.	Thermo tolerance	*OgTT1*	Li *et al.* (2015)
6.	Submergence	*SUBMERGENCE-1* (SUB1) and *SNORKEL* (SK)	Hattori *et al.* (2009); Xu *et al.* (2006)
		MPK3 mediated *SUB1A*	Singh *et al.* (2016)
7.	Low soil phosphorus	*PUP1*	Chin *et al.* (2011)
8.	Restoration fertility	*Rf3 and Rf4*	Pranathi *et al.* 2016
9.	Drought tolerance	OsNAC6, 10, 9 and 5	Fukao and Xiong. (2013)

Quality Improvement

In addition to the yield improvement development of rice varieties having acceptable quality is a priority in a rice improvement programme. Rice eating and cooking quality of rice grain is generally determined by three physicochemical indexes: amylose content (AC), gel consistency (GC) and gelatinization temperature (GT). Markers linked to these traits and progresses in the improvement of quality are given in the table 3.

Table 3: Progress of molecular breeding for quality improvement

S.No	Trait	QTLs/gene	Reference
1.	Amylose content	*Wx*	He *et al*. (1999)
		qAC-6, qAC-5, qAV-4, qAC-3	Li *et al*. (2003)
		amy6-1	Amaravathi *et al*. (2008)
2.	Gelatinization temperature	*starch synthase IIa (SSIIa)*	Umemoto *et al*. (2002)
		qASS-6a, qASS-6b, qASS-3	Li *et al*. (2003)
		qGT-6	Shobha rani *et al*. (2011)
3.	Grain chalkiness	*Chalk5*	Li *et al*. (2014)
		qTGW6	Kim *et al*. (2014)
		OsSPL16/GW8 and GW7	Wang *et al*. (2015b)
4.	Aroma	*Badh2*	Bradbury *et al*. (2005)
		Badh2	Chen *et al*. (2008)
		BADEX7-5	Sakthivel *et al*. (2009)
5.	Grain length/width	*qTGW6*	Kim *et al*. (2014)
		OsSPL16/GW8 and GW7	Wang *et al*. 2012
6.	Grain width and weight	*GW2*	Song *et al*. (2007)
7.	Grain length and weight	*GS3*	Mao *et al*. (2010)
8.	Grain width	*GW5*	Weng *et al*. (2008)
9.	Grain size	*GS2/OsGRF4/GL2*	Che *et al*. (2015)
10.	Grain yield	*OsCKX2*	Ashikari *et al*. (2005)
11.	Kernel Elongation after cooking	*GS3*	Ramkumar *et al*. (2010)

New technology to develop SNP markers

The high demand for sequence data has been driven the development of high throughput sequencing or next generation sequencing technologies that can produce 1000 or millions of sequences concurrently. NGS relies on massively parallel sequencing and imaging techniques to yield several 100s of millions to several 100s of billions DNA bases per run (Shendure and ji, 2008). Several NGS platforms, such as Roche 454 FLX Titanium, Illumina Miseq and Hiseq2500, Ion Torrent PGM have been developed and used recently. NGS technologies have been recently used for whole genome sequencing and re-sequencing projects where genomes of several specimens are sequenced to discover large number of SNPs, construction of haplotype maps and genome-wide association studies. In rice, a core collection of 3,000 rice accessions from 89 countries got sequenced got an average sequencing depth of 14×, with average genome coverages and mapping rates of 94.0% and 92.5%, respectively. From sequencing efforts, approximately 18.9 million single nucleotide polymorphisms (SNPs) were discovered (The 3,000 rice genomes project, 2014). The data serves as a foundation for large-scale discovery of novel alleles for important rice phenotypes using various bioinformatics and/or genetic approaches

Genotype-by-sequencing (GBS) a novel application of NGS protocols for discovering and genotyping SNPs in crop genomics and populations. The recent advances in NGS have reduced the DNA sequencing cost to the point that GBS is now feasible for large genome species and high diversity (Elshire *et al.* 2011). The value of sequencing restriction site associated genomic DNA (RAD) for high density SNP discovery and genotying was demonstrated by Baird *et al.* (2008). It enables the detection of thousands of millions of SNPs in the large collections of lines that can be used for genetic diversity analysis, linkage mapping, GWAS and evolutionary studies.

(Beissinger *et al.* 2013). Recently, GBS through the NGS approach has been used to re-sequence collection of recombinant inbred lines (RILs) to analyze and map various traits of interest in specific breeding programmes (Deschamps *et al.* 2012). It has been used for the genetic diversity studies in rice germplasm. GBS can assist in characterizing the genomic constitution and genetic architecture of MAGIC Population (multi-parent advanced generation intercross) (Huang *et al.* 2015; Pascual *et al.* 2015). Genetic studies on MAGIC populations have revealed quantitative trait loci (QTLs) that control the transition from the vegetative to reproductive stage (Sannemann *et al.* 2015; Meng *et al.* 2016), yield (Huang *et al.* 2015; Pascual *et al.* 2015), grain quality (Bandillo *et al.* 2013), morphological development (Huang *et al.* 2015; Pascual *et al.* 2015; Meng *et al.* 2016), and responses to abiotic and biotic stresses (Bandillo *et al.* 2013). GBS is becoming increasingly important as a cost-effective and unique tool for genomics-assisted breeding in a range of plant species.

Genome-wide Association Studies (GWAS)

With the rapid development of high-throughput sequencing technologies, genome-wide association study (GWAS) has become a new approach for dissecting important agronomic traits of rice. GWAS is a strategy to perform association analysis between agronomically important traits and genotypes using genetically highly diverse germplasms. The genome-wide association approach (GWAS) overcomes several limitations of traditional gene mapping by (i) providing higher resolution, often to the gene level, and (ii) using samples from previously well-studied populations in which commonly occurring genetic variations can be associated with phenotypic variation. The advent of high-density single-nucleotide polymorphism (SNP) typing allowed whole-genome scans to identify often small haplotype blocks that are significantly correlated with quantitative trait variation.

Huang *et al.* (2010) using low depth (1X) whole-genome sequencing, and taking advantage of the >100 kb LD in rice to impute missing data, identified an unbiased set of common SNPs that they used to identify strong associations between genetic loci and various agronomic traits which includes heading date, grain size, and starch quality. This strategy was successful because the imputation algorithm that was developed reduced the missing data from 60% to 3%, with 98% accuracy. GWAS was subsequently performed using 671,355 SNPs in a subset of 373 *indica* lines to avoid the major confounding of population structure between subspecies. This identified between 1 and 7 loci for each agronomic trait, each of which explained between 6% and 68% of the variation in that trait. A few genes that have large effects in controlling traits that are involved in determining yield, morphology, stress tolerance, and nutritional quality were also identified in recent rice GWAS (Famoso *et al.* 2011; Zhao *et al.* 2011). Large-scale GWAS on 38 agronomic traits identified 130 associated loci through developing an integrated genomic approach to construct a genome map for 1,495 elite hybrid rice varieties and their inbred parental lines, which provided a global view of heterosis from a representative number of hybrid combinations (Huang *et al.* 2015). A large number of GWAS peaks associated with panicle trait were identified through quantifying 49 panicle phenotypes in 242 tropical rice accessions with the imaging platform PANorama (Crowell *et al.* 2015). Yano *et al.* (2016) generated a set of 426,337 SNPs and 67,544 InDels by whole-genome sequencing of the 176 varieties, which identified four new genes associated with agronomic traits. Availability of the re-sequencing data of multiple accessions of the same species or different species has initiated the pan-genome (full complement of genes in a given species or population) and generated hapmap information for the construction of high density linkage maps. With the accumulation of genomic sequencing data, high-throughput phenotyping and the perfection of various statistical methods, more and more loci related to agronomically important traits would be identified by GWAS.

Genomic selection

Genomic selection (GS) is an upgraded form of MAS. It aims to use genome-wide markers to estimate the effects of all loci and thereby compute a genomic estimated breeding value (GEBV), so as to achieve more comprehensive and reliable selection. Because the objects of selection are no longer limited to the traits determined by a few major genes, GS

opens up a promising research direction for molecular breeding and it has become a hot issue in recent quantitative genetics research. GS can establish associations between markers and phenotypes based on a training population. The quantitative trait locus (QTL) detection step is skipped in GS, and a prediction model of phenotypic traits and genome-wide markers is constructed. Using this model, the genetic effect values of unobserved individuals are predicted, avoiding the omission of some small-effect markers that would fail a significance test. Even if the effect of each marker is very small, a large amount of marker information covering the whole genome still has the potential to explain all the genetic variance. To perform crop genomic prediction, a large number of loci should be genotyped. In recent years, DNA marker technology has developed rapidly. Markers based on chip technology, such as Diversity Array Technology (DArT) and single-nucleotide polymorphisms (SNP have laid a strong foundation for the application of GS in crop breeding. When applied to hybrid breeding of crops, GS is even more efficient because genotypes of hybrids can be inferred from their inbred parents, leading to lower cost in genotyping (Kadam *et al.* 2016; Beukert *et al.* 2017). Xu *et al.* (2014) used 278 randomly selected rice hybrids derived from 210 recombinant inbred lines (RIL) as a training set and predicted 21,945 potential hybrids. The average yield of the top 100 showed a 16% increase compared with the average yield of all potential hybrids. Gartner *et al.* (2009) showed that the predictive power of biomass heterosis for hybrids in rice was significantly improved with parental metabolic measurements. In short, integrating multiple omic data is expected to be an important method for the study of GS in crop breeding. Together, these studies establish a research platform that can link genomic variation and germplasm collections to enable molecular breeding.

Allele Mining and TILLING

While lot of progress in molecular breeding in terms of development of improved varieties by accumulation of beneficial alleles from known donors, still, a significant portion of these beneficial/superior alleles were not utilized as these were left behind during evolution and domestication. This untapped genetic variation existing in wild relatives and land races of crop plants could be exploited gainfully for development of agronomically superior cultivars. Introgression of novel alleles from wild relatives of crop plants into cultivated varieties (deVicente and Tanksley, 1996; Xiao *et al.* 1996; McCouch *et al.* 2007) have clearly demonstrated that certain alleles and their combinations potentially make dramatic changes in trait expression when moved to a suitable genetic background by overcoming the genetic bottlenecks which restricted their introgression to cultivars. Hence, the vast rice germplasm resources need to be relooked for novel alleles to further enhance the genetic potential of rice for various agronomic traits. Enormous progress has been made in the last 15 years in depositing an exponential amount of sequence information into GenBank (Chan, 2005; Mardis, 2008). With rapid accumulation of sequence and expression data in various genomic databases, identification of useful alleles for various genes from a wide range of species is relatively easy than before. This capability enables direct access to key alleles conferring resistance to biotic and abiotic stresses, greater nutrient use efficiency, enhanced yield and improved quality. Allele mining can be effectively used for discovery of superior alleles, through 'mining' the gene of interest from diverse genetic resources. It can also provide insight into molecular basis of novel trait variations and identify the nucleotide sequence changes associated with superior alleles. Allele mining may also pave way for molecular discrimination among related species, development of allele-specific molecular markers, facilitating introgression of novel alleles through MAS or deployment through genetic engineering (Ramkumar *et al.* 2010).

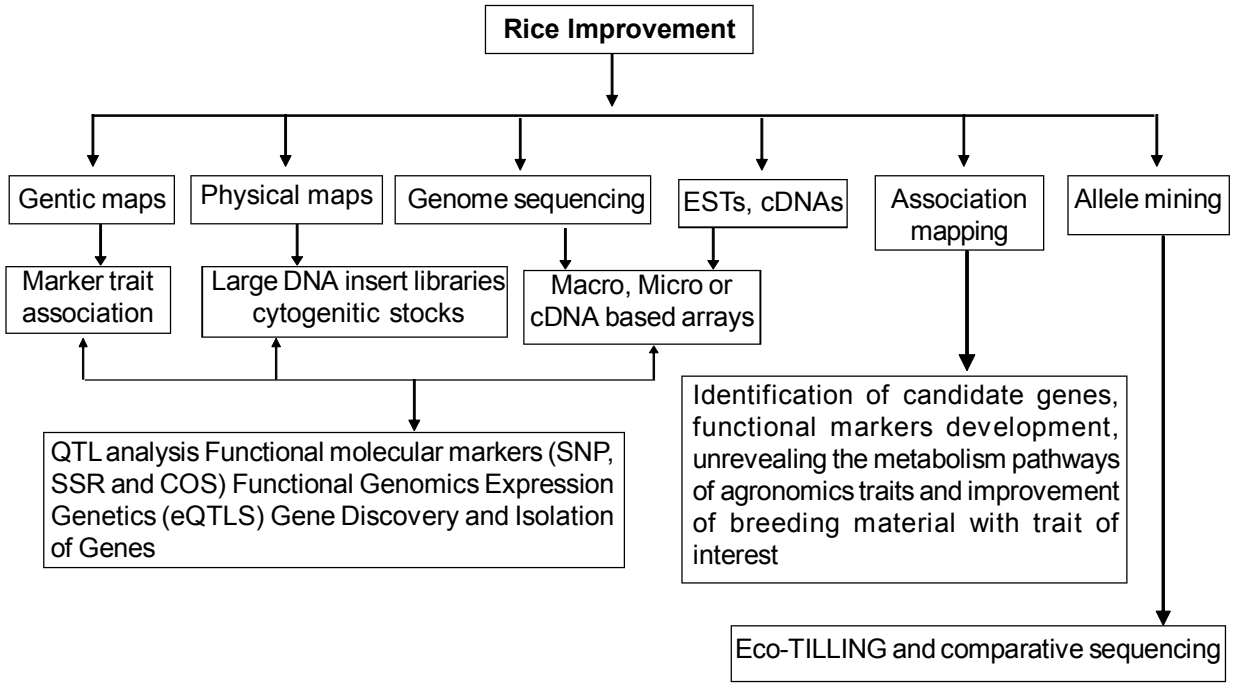
Allele mining of blast resistance, novel or superior alleles of *Pita* gene were reported in wild species and landraces by Ramkumar *et al.* (2010), Sharma *et al.* (2010), Thakur *et al.* (2013). Allele mining of *Pi54* gene was also reported by Sharma *et al.* (2010), Vasudevan *et al.* (2015), Ramkumar *et al.* (2016). In *Piz(t)* the novel alleles were identified by Sharma *et al.* (2010) and Thakur *et al.* (2013). Liu *et al.* (2011) reported novel alleles of *Pi9* in *Oryza* species. In *Pid3* gene novel alleles were identified by Shang *et al.* (2009), Xu *et al.* (2014). Ramkumar *et al.* (2011) developed allele specific InDel marker, *Pi54* MAS for *Pi54* gene. Devi *et al.* (2015) reported NMSMPi-9 and MSM6 allele specific markers for *Pi9* and *Pi40* genes. Tian *et al.* (2016) developed allele specific markers for *Pi2* and *Pi9* genes. Allele mining also done for two major aroma causing genes i.e., *badh2* and *badh1* from the diverse aromatic accessions belongs to different states of India and correlation of alleles structures to the 2-AP content was made (Peddamma *et al.* 2018).

Another strategy for linking a gene with phenotype, i.e., Targeting Induced Local Lesions in

Genomes (TILLING) has been developed. It is a reverse genetic method that combines chemical mutagenesis with high-throughput genome-wide screening for point mutation detection in genes of interest. Eco-TILLING is a method that uses TILLING techniques to look for natural mutations/alleles at a locus to be characterized across germplasm which enables SNP discovery and haplotyping. This method is expected to provide a series of alleles for those genes that are involved in important processes of the plant even though known variants for these genes have not been observed through genetic studies. In case of EMS-induced spotted leaf mutation (spl30) indicated the presence of SNPs membrane transporter genes, *OsAKT1, OsHKT6, OsNSCC2, OsHAK11* and *OsSOS1* exhibited varied levels of expression and tolerance to salt treatments (Hwang *et al.* 2016). This mutant based reverse genetic approach may shift the perception of genetic resources in agricultural studies, because it brings about not only loss of function mutants with various degrees of impairment but also provides gain of function mutants (Piron *et al.* 2010). These characters of TILLING for producing and identifying allelic series of mutations are very important for crop improvement.

Omics-assisted breeding: novel tools for rice improvement

Rice has been recognized as a model for plant research due to its small genome size, accurate genome sequences characterized by co-linearity with the sequences of other cereal crops, high-efficiency transformation technology and abundant germplasm resources (Jiang *et al.* 2012). Rice has been chosen as the model cereal its small genome, the ease with which it can be transformed, its well understood genetics with detailed genome physical maps and dense molecular markers, and the existence of great similarities in gene sequence, gene structure, gene order and gene function among all the cereals and grasses. Genes identified in rice as being important agronomically are also important in other cereals, and any understanding of rice genes is directly applicable to other cereals. Rice is rich in germplasm resources, consists of 21 wild and 2 cultivated species, which are classified into 10 different genome types (Vaughan *et al.* 2003). After the completion of whole genome sequencing in rice, an impressive number of advances in genomics research generating new tools, including collection of germplasm resources, gene expression microarrays, full-length cDNA libraries, generation of mutant libraries and RNA-Seq technologies for expression profiling (Jiang *et al.* 2012; Yang *et al.* 2013b). Platforms of metabolomics, proteomics and phenomics and corresponding databases and bioinformatic tools have also been gradually established and improved in rice (Rajasundaram and Selbig, 2016). The genomes of many germplasm have been re-sequenced with the development of next-generation sequencing (NGS) technologies (Figure 1).



Transcriptomics is the study of the complete set of RNA transcripts that are produced by the genome, under specific circumstances or in a specific cell. There are two key contemporary techniques; microarrays, which quantify a set of predetermined sequences and RNA-Seq, which uses high-throughput sequencing to record all transcripts. However, use of these technologies for applied aspects in plant breeding has been limited because, expect for NILs, differential gene expression is caused not only by the trait of interest but also by the variation present in the genetic background. Therefore, background effects must be eliminated to establish a functional association between the level of gene expression and a given trait. Wang *et al.* (2014c) used an Affymetrix Gene Chip Rice Genome Array to analyze the expression QTLs (eQTLs) in rice seedlings and flag leaves during heading period from recombinant inbred lines (RILs) derived from a cross between Zhenshan 97 and Minghui 63. They found a large number of *cis*- and *trans*-eQTLs that regulate the expression of genes, leading to the construction of the regulatory network through gene co-expression analysis (Wang *et al.* 2014c). Further analysis of the flag leaves of 98 immortalized F_2 (IMF2) identified many genomic loci that control the expression abundance of small RNAs (Wang *et al.* 2015a), providing new insight into the regulation of gene expression.

For rice functional genomics, the construction of high-throughput and accurate phenomics platforms is becoming a new research area. Australian Plant Phenomics Facility has successfully applied this technology in the studies of salt stress (Rajendran *et al.* 2009), drought tolerance (Normanly, 2010), toxicity (boron) tolerance (Schnurbusch *et al.* 2010), as well as modeling and prediction of crop yield (Golzarian *et al.* 2011) and root development (Rahnama *et al.* 2011). Huazhong Agriculture University, in collaboration with Huazhong University of Science and Technology, developed a high-throughput rice phenotyping facility (HRPF), which allows the growing of 5,472 pots of rice and can automatically monitor 15 important agronomic traits (Yang *et al.* 2013a; Yang *et al.* 2014).

Breeding by Design

Knowledge of the map positions of all loci of agronomic interest, the allelic variation at those loci, and their contribution to the phenotype should enable the breeder to design superior genotypes comprising a combination of favourable alleles at all loci. Because the positions of all loci of importance are mapped precisely, recombination events can be accurately selected using flanking markers to collate the different favourable alleles next to each other. Software tools should enable us to determine the optimal route for generating those mosaic genotypes by crossing lines and using markers to select for the specific recombinants that will eventually combine all those alleles. Because this is a precisely defined process, selection by phenotyping can be omitted. Only the eventually obtained superior varieties will be evaluated for field performance.

Advances in genetic engineering

Introduction of resistance in rice to insect pests has always been an important endeavour. Yield losses mainly occur due to the infestation of stem borers and estimated to be 5-10% (Pathak and Khan, 1994). Conventional plant breeding approaches for developing resistant varieties were not successful due to non-availability of effective sources of resistance against Yellow stem borer (*Scirpophaga incertulas*), Leaf folder (*Cnaphalocrocis medinalis*) and Striped stem borer (*Chilo suppressalis*). Many useful insecticidal proteins and respective genes have been identified and isolated from plants, animals and from microorganisms (Kumar *et al.* 1996). Among these proteins, a class of insecticidal proteins found in a soil bacterium *Bacillus thuringiensis* (Bt) assumed significance and wide commercial application in crop species such as cotton, maize and potato (Kumar *et al.* 1996). Bt toxins are highly specific against insects pests without affecting predators and other beneficial insects (Kumar *et al.* 1996). These Bt toxins kill susceptible insects feeding on the crop parts/tissues. This means that Bt crops are especially useful for controlling pests that feed inside plants and that cannot be killed readily by sprays such as the Yellow stem borer, which bores into stems. Bt genes have also been successfully introduced and expressed in different rice varieties for the management of stem borers. Transgenic insect resistant rice developed using these genes have been tested under field conditions, which showed resistance and yield advantage. The most frequently used Bt genes are

Cry1A, Cry1Ab and *Cry1Ac* and *Cry1Ab/ Ac* fusion gene.The progress on development of transgenics for various traits using genetic engineering tools are given in table 4.

Table 4: Successful examples of development of transgenics for various traits

Biotic stress			
Insect pest			
S.No	**Protein/Gene**	**Cultivar**	**Reference**
1	*Cry1Ab*	KMD1, KMD2	Shu *et al.* (2000)
2	*Cry1Ac*	Elite Eyi 105	Loc *et al.* (2002)
3	*Cry1Ac*	IR64, Pusa Basmti-1, Karnal Local	Khanna *et al.* (2002)
4	*Cry1Ab*	IR58	Wunn *et al.* (1996)
5	*Cry1Ab*	IR72, IR64, CBII, Taipei-309, IR 68899 B, Mh-63-63	Datta *et al.* (1998)
6	*Cry1Ac*	Tarom Molaii	Ghareyazie *et al.* (1997)
7	*Cry1Ac*	IR64	Nayak *et al.* (1997)
8	*Cry2A*	Basmti-370, M-7	Sheng *et al.* (2003)
9	*Cry1B*	Ariete, Senia	Breitler *et al.* (2000)
10	*Cry1Ab/ Cry1Ac*	CMS restorer, Minghui63, Shanyou 63	Tu *et al.* (2000)
11	*OsLecRK1–OsLecRK3*	Rathu Heenati	Liu *et al.* (2015)
12	Onion lectin/ Snowdrop lectin	Chaitanya and BPT 5204	Bharathi *et al.* (2011)
Diseases			
1	*Xa21* for blight resistance	IR72	Tu *et al.* (2000); Laha *et al.* (2003)
2	Cecropin B peptide for blight resistance	Nipponbare	Sharma *et al.* (2000)
Blast			
3	*Gns1*	Nipponbare	Nishizawa et al. (2003)
4	*Cht-2* or *Cht3*	Nipponbare and Khoshihikari	Nishizawa *et al.* (1999)
5	*afp* gene	Senia	Coca *et al.* (2004)
6	*OsJAMyb*	Heikezijing	Cao *et al.* (2015)
7	*OsWRKY13*	Nipponbare	Quilis *et al.* (2008)
8	*OsNPR1, 2* and *3*	Nipponbare	Fitzgerald *et al.* (2004)
9	*OsAOS2*	Nipponbare	Mei *et al.* (2006)
Sheath blight			
10	*chi11*	Pusa Basmati-1	Sridevi *et al.* (2008)
11	Endochitinase gene (*cht42*)	Pusa Basmati-1	Shah *et al.* (2009)
12	*npr* for resistance to blast, sheath blight and blight diseases	Chaitanya	Sadumpati *et al.* (2013)
13	The coat protein (CP) gene for rice stripe virus resistance.	Nipponbare and Kinuhikari	Hayakawa *et al.* (1992)

S.No	Protein/Gene	Cultivar	Reference
14	Ribozyme-mediated resistance to rice dwarf virus	Tongling No.1	Han *et al.* (2000)
15	spike protein for rice ragged stunt virus resistance	Jarrah	Chaogang *et al.* (2003)

S.No	Protein/Gene	Pathway/ Function	Reference
Abiotic Stress			
1.	*OsbZIP72*	ABA response	Lu *et al.* (2009)
2.	*TOND1*	Tolerance of Nitrogen Deficiency	Zhang *et al.* (2015)
3.	*NRT1.1B*	Higher nitrogen-use efficiency	Hu *et al.* (2015a)
4.	*Pup1*	phosphorous up take Efficiency	Chen *et al.* (2011)
5.	*OsPTF1(*from Kasalath)	phosphorous up take Efficiency	Yi *et al.* (2005)
6.	*â-carotene Synthase*	â-carotene Synthesis	Paine *et al.* (2005)
7.	*Ferritin (From Phaseolus vulgaris)*	Bioavailability of iron	Aluru *et al.* (2008)
8.	*Phytase(*from *Aspergillus fumigates)*	Bioavailability of iron	Aluru *et al.* (2008)
9.	*H+ /Ca2+ transporter gene*	Increase calcium content	Kim *et al.* (2005)
10.	*Bar(Alcaligenes faecalis)*	Herbicide Tolerant	Oard *et al.* (1996)
11.	*EPSP synthase(from Agrobacterium tumefaciens)*	Herbicide Tolerant	Chhapekar *et al.* (2015)
12.	*MerA (*bacteria)	phytoremediation of mercury	Heaton *et al.* (2003)
13.	*SQS*	ABA synthesis	Manavalan *et al.* (2011)
14.	*OsDST*	ABA sensing	Huang *et al.* (2009)
15.	*JERF1*	AP2/ERF family transcription factor	Zhang *et al.* (2009)
16.	*TSRF1*	AP2/ERF family transcription factor	Quan *et al.* (2010)
17.	*OsDREB2A*	AP2/ERF family transcription factor	Mallikarjuna *et al.* (2011)
18.	*ZmCBF3*	AP2/ERF family transcription factor	Xu *et al.* (2011)
19.	*OsNAC5*	NAC family transcription factor	Takasaki *et al.* (2010)
20.	*OsZFP245*	Protein degradation	Huang *et al.* (2009)
21.	*OsSIK1*	Protein degradation	Huang *et al.*, (2009)
22.	*OsWRKY11*	Protein degradation	Ouyang *et al.* (2010)
23.	*OsD1S1*	Protein degradation	Park *et al.* (2010)
24.	*OsD1R1*	Protein degradation	Ning *et al.* (2011)
25.	*IPT*	Cytokinin Biosynthesis	Peleg *et al.* (2011)
26.	*OsbHLH148*	Jasmonate signalling	Seo *et al.* (2011)

S.No	Protein/Gene	Pathway/ Function	Reference
27.	*OsSKIP1*	Stress response	Hou *et al*. (2009)
28.	*OsDHODH1*	Pyrimidine nucleotide biosynthesis	Liu *et al*. (2009)
29.	*cspA, cspB*	RNA chaperones	Castiglioni *et al*. (2008)
30.	*OSnac10*	Transcription factor	Mittler *et al*. (2006)
31.	*Los 53*	ABA synthesis	Jeong *et al*. (2010)

New Tools for rice improvement

Activation Tagging for deciphering gene function

Activation tagging is a powerful gain-of-function approach to reveal the functions of genes, especially those with high sequence similarity recalcitrant to loss-of-function genetic analyses. Activation tagging randomly inserts a T-DNA fragment containing engineered four copies of enhancer element into a plant genome to activate transcription of flanking genes. The multiple rice mutant libraries generated by T-DNA insertion, *Ds/dSpm* tagging, *Tos17* tagging, and chemical/irradiation mutagenesis, have been developed. A total of 2,46,566 flanking sequence tags from rice mutant libraries with T-DNA, *Ds/dSpm*, or *Tos17* insertion were obtained, targeting 2,11,470 unique sites (Yang *et al.* 2013a). Currently, 57% of non-transposable-element-related genes in rice have insertion tags (Jiang *et al.* 2012).These resources include several functions such as knockout, gene trap, enhancer trap, and/or activation-tag. Most of the flanking sequence tags (FSTs) are searchable at the RiceGE (http://signal.salk.edu/cgi-bin/RiceGE) and OrygenesDB (http://orygenesdb.cirad.fr) websites. Collectively, all these mutant resources are of great value for both functional genomics and genetic improvement in rice.

Genome assisted mutagenesis

Thus, generation of large-scale mutants at the whole-genome level is of great value for both functional genomics and genetic improvement of rice. Traditionally, large numbers of mutants are produced by physical, chemical, or biological mutagenesis. Mutants created by these methods have made enormous contributions to basic plant research and crop improvement. There are 3 ways in which to induce mutations, by either using: 1) biological agents such as transposons and T-DNA, 2) physical agents such as fast neutron, UV and x-ray radiation, or 3) chemical agents such as N-methyl-N-nitrosourea (MNU),1,2:3,4-diepoxybutane (DEB) or ethyl methanesulfonate (EMS) and can efficiently screen following Targeting Induced Local Lesions IN Genomes (TILLING) high-throughput screening protocols. Induced mutations can be efficiently integrated with genomics, transcriptomics, and proteomics and metabolomics studies to understand the phenome. Populations of induced mutants are useful because their genetic variation is typically superimposed on uniform genetic background. Once mutations are identified in a specific gene of interest, researchers can acquire seeds representing the next generation and investigate their phenotypic consequences. An approach combining analysis of several lines carrying independent mutations in the same gene and repeated backcrosses to "clean" each line of background mutations is required before conclusions can be reached as to the actual link between phenotype and mutation genotype. In the popular rice variety, IR64 background, mutation has been reported by using 1.6% EMS (Wu *et al.* 2005). Similarly, in Nagina 22, a total of 22,292 mutagenized lines have been generated, which are in different generations and stages of phenotypic evaluation (Mohapatra *et al.* 2014). Recently IIRR in collaboration with CCMB developed the 10, 500 mutant lines in the back ground of Samba Mahsuri and identified the resistant mutant to the sheath blight, yellow stem borer ,early maturing and mutant having high yielding characters (Gopi *et al.* 2014). The potential mutants are being used for identifying the concerned loci cussing the phenotype through genomics.

RNA interference (RNAi)

RNA interference (RNAi) is a promising gene regulatory approach in functional genomics that has significant impact on rice improvement which permits down-regulation in gene expression with greater precise manner without affecting the expression of

other genes. In RNA interference, the post transcriptional gene silencing (PTGS) trigger by double stranded RNA (dsRNA) molecules to prevent the expression of specific genes. This promising approach also imparts its effective and efficient role to knock down the expression of any particular gene through small interfering RNA (siRNAs) molecules in any target cell and moreover to assess the changes that occur in signaling pathways. Introduction of short pieces of double stranded RNA (dsRNA) and small interfering RNA (siRNA) into the cytosol, initiate the pathway culminating targeted degradation of the specific cellular mRNA. During RNAi mechanism, silencing initiate with enzyme Dicer and dsRNA is processed to convert the silencing trigger to ~22-nucleotide, small interfering RNAs (siRNAs). The antisense strand of siRNA become specific to endonuclease-protein complex, RNA-induced silencing complex (RISC), which targets the homologous RNA and degrade it at specific site that results in the knock-down of protein expression (Kola *et al.* 2016). RNAi has also been exploited in plants for resistance against pathogens, insect/pest, nematodes, and virus that cause significant economic losses.

RNA-interference (RNAi) is involved in several biological phenomena, including resistance against pathogens, insect/pest, nematodes, and virus that cause significant economic losses (Yu and Kumar, 2003;Herr, 2005). RNAi can knockdown *OsSSI2* (*OsSSI2*-kd), meant for fatty acid desaturase activity that cause increase resistance against bacterial blight and blast diseases in rice (Jiang *et al.* 2009). For Yellow Stem borer, dsRNA designed from *Cytochrome P450* and *Aminopeptidase N* has showed detrimental effect on larval growth and development (Kola *et al.* 2016). In relation to drought responses, among genetically engineered plants the rice exhibited gene expression of RACK1 inhibition caused by RNAi, which explained the potential role of RACK1 to drought stress and observed a superior level of tolerance in contrast to non-transgenic rice plants (Jian *et al.* 2010). The rice starch branching enzyme (RBE3 gene) plays a critical role in rice grain starch synthesis. The silencing of RBE3 gene improves amylose content in rice and indicated that amylose content was negatively correlated with BEII activity, spike size, and TGW (Jiang *et al.* 2013).

Genome editing

Genome editing (GE) is a method that enables specific nucleotides in the genome of an individual to be changed. The recent emergence of genome editing technologies has superseded the limitations of traditional breeding methods starting a new era of crop improvement. Genome editing involves the usage of engineered site-specific nucleases (SSNs) to modify specific genes at desired locations in the genome. The SSNs such as zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR)-associated endonuclease Cas9 (CRISPR/Cas9) make a double-stranded break (DSB) in the target DNA which is subsequently repaired by cell's own natural repair mechanism of homologous recombination (HR) or non-homologous end joining (NHEJ) (Miglani, 2017). The NHEJ repair is the error prone pathway which creates random insertions and deletions (indels) and results in frame shift mutations and targeted gene knockouts (Feng *et al.* 2013; Bortesi and Fischer, 2015), whereas the HR pathway is much more precise in the exchange of homologous sequence leading to gene knock in or gene replacement (Voytas and Gao, 2014; Baltes *et al.* 2014).

Clustered regularly interspaced short palindromic repeats-associated endonuclease Cas9 is the most advanced genome editing tool in plant biology (Belhaj *et al.* 2015; Weeks *et al.* 2016). It consists of a short RNA molecule called guide RNA which is associated with a DNA endonuclease called Cas9. CRISPR-associated protein 9 (Cas9) is a DNA endonuclease responsible for cutting the invading phage DNA into pieces, which then gets integrated into the CRISPR array as a spacer. Several disease related genes have been mutated in the recent times using the genome editing approaches to increase disease resistance in rice. Transcription activator-like effectors (TALEs), the type III effector proteins from *Xanthomonas* species, usually target the SWEET gene family, the sugar transporters that release the sugar into the apoplast of rice cells (Cohn *et al.* 2014). TALEN technology was used to disrupt the bacterial protein binding sequence in the promoter of *OsSWEET14* for conferring resistance against bacterial blight (Li *et al.* 2012). Similarly, CRISPR/

Cas9 technology was used to construct a null mutation in *OsSWEET13* to prevent its neutralization by the TAL effector gene *pthXo2* leading to improved resistance toward bacterial blight disease in *indica* rice, IR24 (Zhou *et al.* 2015). Most recently, TALEN technology was used to modify the *EBEtal7* binding site in the *Os09g29100* gene promoter to reduce Tal7 binding, which could potentially reduce BLB disease severity in rice (Cai *et al.* 2017). CRISPR/Cas9-targeted knockout of ERF transcription factor gene *OsERF922* has demonstrated enhanced resistance to rice blast (Wang *et al.* 2016). CRISPR/Cas9-mediated editing of *eIF4G* gene has been reported in the RTSV susceptible rice variety, IR64 as an attempt to develop new source of resistance to RTD (Macovei *et al.* 2018). The RTSV-resistant plants with the novel *eIF4G*alleles can be used as valuable materials to develop more diverse RTSV-resistant varieties. Recently, genome editing-based mutations have been introduced within the *ALS* gene to produce herbicide tolerant rice varieties (Li T. *et al.* 2016; Sun *et al.* 2016). CRISPR/Cas9 technology was also employed to edit the *TIFY1b* and its homology gene *TIFY1a* (Huang *et al.* 2017) for inducing cold tolerance. Recently, a genome-wide mutant library has been generated using CRISPR/Cas9 (Lu *et al.* 2017; Meng *et al.* 2017).

Conclusion

Application of biotechnological tools has resulted in remarkable progress in understanding the genetic and molecular basis of various agronomically important traits in rice paving the way for furthering our food and nutritional security. The exploitation of the twin tools of marker-assisted breeding and genetic engineering could have a great impact on rice improvement. The recent integration of advances in molecular biology, genomic research, transgenic breeding and molecular marker applications with conventional plant breeding practices has created the foundation for molecular rice breeding or 'precision' breeding in rice. In recent years, genome editing has come of age and is presumed to revolutionize crop improvement. Through a judicious application of all the modern tools and techniques of biotechnology, development of a designer rice plant which is high yielding, nutrient-use efficient, tolerant to biotic and abiotic stresses and with enhanced nutritional quality and resilient to climate changes should be possible in the near future.

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Research Article
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CHARACTERIZATION AND SCREENING OF SALINITY TOLERANT POTASSIUM SOLUBILIZING BACTERIA

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ABSTRACT

Salinity stress is one of the major abiotic threats to plant life and agriculture worldwide and significantly reduces crop yield in affected areas. Excessive salinity limits plant growth and productivity and can lead to plant death. Soil salinity in arid regions is frequently an important limiting factor for cultivating agricultural crops. Potassium deficiency frequently compounds the problems of saline soil. Salt tolerant Potassium Solubilizing Bacteria (KSB) reduced the impact of salinity on plant growth and improved productivity by solubilizing unavailable potassium in the soil. In the present study native KSB was isolated from saline soils of Telangana, and the isolates were culturally, morphologically and biochemically characterizated of which 4 were identified as *Bacillus* sp., 3 as *Pseudomonas* sp. and 2 as *Azotobacter* sp.according to the Bergey's manual of systemic bacteriology. The isolates were screened for ACC deaminase activity, EPS production and PGP attributes like siderophore production, HCN and IAA production. Out of 9 isolates, 4 produced ACC deaminase, 6 isolates produced exopolysaccharides 4 produced HCN and 6 isolates showed positive for IAA production and only 2 isolates produced siderophores.

Potassium is the third important plant nutrient and is essential macronutrient for plant growth and plays significant role in activation of several metabolic processes including protein synthesis, photosynthesis, enzyme activity, as well as in resistance to diseases and insects etc., (Rehm and Schmitt, 2002). Potassium though present as abundant element in the soil or is applied to fields as natural or synthetic fertilizers, only one to two per cent of this is available to plants, the rest being bound with other minerals and therefore unavailable to plants. The most common soil components of potassium *i.e.,* 90 to 98 % are feldspar and mica (Mcafee, 2008). Very little of this potassium source is available for plant use. Silicate bacteria were found to resolve potassium, silicon and aluminum from insoluble minerals (Alexander, 1985). Their uses as biofertilizers or biocontrol agents for agriculture improvement and environmental protection have been a focus of recent research. Certain bacteria are capable of decomposing alumino silicate minerals and releasing a portion of the potassium contained therein (Biswas and Basak, 2009).Soil microorganisms influence the availability of soil minerals, playing a central role in ion cycling and soil fertility (Lian *et al.,* 2010).

Potassium deficiency frequently compounds the problems of saline soil. High salinity affects plant growth through many destructive effects such as an osmotic effect and harmfulness of salt ions, as well as variations in the biological and chemical properties of soil. It also suppresses potassium uptake by plant roots and reduces the available potassium by absorption processes and low solubility of the K mineral. Since potassium is a critical nutrient influencing plant growth, this adversely affects plant growth under stress conditions (Keren, 2000). Nowadays application of inorganic fertilizers is the most common approach to improve soil fertility. However, the application of potassium supplements in chemical fertilizers is quickly fixed to the insolubilized forms, especially in saline soil, and this is the reason for low availability of potassium. Recent studies have proved that the use of biofertilizer combined with 25 % of chemical fertilizer gives good results for plant growth in the long term (Kramany *et al.,* 2007).

Potassium solubilizing rhizobacteria, such as *Acidothiobacillus* sp*., Bacillus edaphicus*, *B.mucilaginosus, Ferrooxidans* sp., *Pseudo-*

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monas sp., *Burkholderia* sp. and *Paenibacillus* sp., have been reported to release potassium in accessible form from potassium bearing minerals in soils (Liu *et al.,* 2012). Thus, applying potassium solubilizing PGPR as biofertilizer to improve agriculture can reduce the use of agrochemicals and support ecofriendly crop production (Setiawati and Mutmainnah, 2016). Potassium solubilization by Potassium Solubilising Microorganisms (KSM) is mainly due to the production of organic acids (Han and Lee, 2005; Meena *et al.,* 2013). The use of microbial inoculants as biofertilizers is a sustainable agriculture practice for providing an alternative for chemical fertilizer by using this farmers can mobilize the potassium present in their own soil and save some percentage of their potassium fertilizer requirement. The potassium solubilizing bacteria may prove a useful tool in developing approaches to enable plant growth in saline soils. Therefore, the application of salinity tolerant KSM would not only counter balance the high production cost of potassium fertilizers, but also transform the insoluble potassium in the soil or in fertilizers applied. The reports on salt tolerant KSM are found to be very few, so the present study was taken up to isolate, screen and identify the potential salt tolerant P solubilizing rhizobacteria from the saline rhizospheric soils of Telangana.

MATERIAL AND METHODS

Soil sampling

Saline soils have been identified in Telangana and samples were collected from different saline rhizospheric soils of Mahabubnagar,Nalgonda and Rangareddy district of Telangana state. Rhizospheric soil samples were selected based on salinity and sampling was done to a depth of 10 to 15 cm. The plant was uprooted and the soil intimately adhering to the roots was collected and mixed to provide a composite soil sample. All the samples were separately bagged, labelled, air dried and stored in a refrigerator at 4 ^{0}C for further studies.

Isolation of PGPR

For isolation of rhizobacteria the method proposed by Vlassak *et al.* (1992) was followed. In this procedure 10 g of soil from each soil sample was taken in a conical flask to which 90 ml of normal saline (0.85 %) was added. The sample was agitated for 15 minutes on a vortex and serial dilutions of soil suspensions were prepared. From the dilutions 0.1 ml of sample was spread on sterilized petri plates containing Trypticase Soy Agar (TSA) media with 2 % NaCl and the petri plates were incubated at room temperature (28 ^{0}C ± 2 ^{0}C) for 24 - 72 h. Two replicates were maintained for each dilution and the plates were examined daily up to 3 days for bacterial colonies. The colonies were purified and evaluated for salinity tolerance.

Plate assay for potassium solubilization

The isolates were spot inoculated on Aleksandrov's agar medium constituted with 0.5 % potassium aluminium silicate and incubated at 30 ^{0}C for 48 h. The diameters of the clearing zones around the colonies were measured (Sugumaran and Janartham, 2007).

Screening for salinity tolerance

Trypticase soy agar medium plates were prepared with different salt concentrations *i.e.,*2 %, 5%, 10% and 15%. All the isolates were spot inoculated on these prepared plates. For each treatment three replications were maintained. The petriplates were incubated at 28 ± 2 ^{0}C for 24 - 48 h. Based on visual observations, cultures showing best growth on different concentrations of salts were chosen.

Characterization of the isolates

The bacterial isolates were identified on the basis of morphological, cultural, physiological and biochemical characteristics according to the standard methods described in Bergey's manual of systematic bacteriology (Holt *et al.,* 1984).

All the isolated bacterial cultures were grown and purified by the streaking as single colony in the TSA medium and allowed to grow for 24 - 48 h and the isolates were grown on different mediums like nutrient agar (NA), king's B agar (KB), Waksman's 77 agar & Yeast Extract Mannitol Agar with congo red (YEMA) medium for further confirmation of organisms. Pure cultures were preserved at 4 ^{0}C for further studies.

Biochemical and physiological characterization

Different biochemical tests were performed using standard procedures. The protocols followed are briefly outlined below.

Indole production

Sterilized hydrogen sulfideindole motility agar slants were inoculated with the overnight cultures of the isolates and incubated for 48 h at 28 ± 2 ^{0}C. Following incubation, 10 drops of Kovac's indole reagent was added to each tube. The isolates showing production of red colour was recorded as positive for indole production (Isenberg and Sundheim, 1958).

Catalase test

This test was performed to study the presence of catalase enzyme in bacterial colonies. Pure isolates (24 h old) were taken on glass slides and one drop of H_2O_2 (30 %) was added. Appearance of gas bubble indicated the presence of catalase enzyme (Rangaswami and Bagyaraj, 1993).

Oxidase test

The overnight grown cultures of the test isolates were spotted on plates poured with sterile Trypicase Soy Agar and the plates were incubated for 24 h at 28 ± 2 ^{0}C. After incubation, 2 - 3 drops of N, N, N', N'- tetramethyl- p-phenylene diaminedihy drochloride (Wurster's reagent) was added on to the surface of growth of each test organism. The isolates showed change of colour to maroon were noted as positive (Collins and Lyne, 1970).

Gelatin liquefaction

The overnight cultures of the test isolates were inoculated to sterilized nutrient gelatin deep tubes and incubated for 24 h at 28 ± 2 ^{0}C. Then the tubes were placed in the refrigerator for 30 minutes at 4 ^{0}C. The isolates showed liquefied gelatin were taken as positive and those which resulted in solidification of gelatin on refrigeration was recorded as negative for the test (Macfaddin, 2000).

Methyl red test

Sterilized glucose phosphate broth tubes were inoculated with the test culture and incubated at 28 ± 2 ^{0}C for 48 h. After incubation five drops of methyl red indicator was added to each tube and gently shaken. Development of red colour indicated positive for the test while yellow colour production indicated negative for the test (Macfaddin, 2000).

Voges-prausker's test

To the presterilized glucose-phosphate broth tubes, test cultures were inoculated and incubated at 37 ^{0}C for 48 h. After incubation ten drops of baritt's reagent A was added and gently shaken followed by addition of 10 drops of baritt's reagent B. Pink colour development in the broth was taken as positive for the test (Macfaddin, 2000).

Citrate utilization

Isolates were streaked on simmon's citrate agar slants and incubated at 28 ± 2 ^{0}C for 24 h. Change in colour from green to blue indicated positive reaction for citrate utilization (Macfaddin, 2000).

Starch hydrolysis

Sterile starch agar plates were spotted with 10 µl overnight broth cultures of the isolates and incubated at 28 ± 2 ^{0}C for 24 - 48 h. After incubation, the plates were flooded with iodine solution. The formation of a transparent zone around the colony was taken as positive for the test (Macfaddin, 2000).

Hofer's alkaline agar test

The isolates were streaked on hofer's alkaline media plates and incubated at 28 ± 2 ºC for 48 - 72 h. *Rhizobium* does not grow on the media plates (Vincent, 1970).

Screening of the isolates for PGP attributes

Siderophore production

Production of siderophores was estimated qualitatively on aqueous ferric chloride solution for siderophores detection. 60.5 mg CAS (chrome azurolsulfonate) was dissolved in 50 ml of distilled water and mixed with 10 ml of iron (III) solution (1 mM $FeCl_3$. $6H_20$ in 10 mMHCl). This was added to 72.9 mg of hexadecyltrimethyl ammonium bromide (HDTMA) in 40 ml of distilled water. The dark blue colored CAS reagent was then autoclaved for 15 minutes. This reagent was added to PIPES agar medium (30.24 g of PIPES buffer dissolved in 750 ml of distilled water +15 g of agar, whose pH was adjusted to 6.8 using 1.06 g of NaOH pellets). The medium is allowed to solidify in the plates and all the isolates were spot inoculated on these prepared plates and incubated at optimum temperature for 3 - 4 days (Schwyn & Neilands, 1987).

Hydrocyanic acid production (HCN)

The HCN production was tested by the method of Castric and Castric (1983). Medium plates *i.e.*, modified nutrient agar was prepared by adding 4.4 g per litre of glycine separately. One ml of culture of each test isolate was inoculated on respective media plates. A disc of whatman filter paper no.1 of the diameter equal to the petri plate size, impregnated with alkaline picric acid solution (0.5 % picric acid (w/v) in 1 % sodium carbonate) was placed in the upperlid of the inoculated petri plates under aseptic condition. The control plate did not receive the inoculum. The plates were incubated upside down at 28 ± 2 ^{0}C for 48 - 72 h. Change in colour from yellow to light brown, moderate or strong reddish brown was taken as indication of HCN production.

Indole acetic acid (IAA) production

Indole acetic acid production was quantitatively measured by the method given by Gordon and Weber (1951). Bacterial cultures were grown in a Luria-Bertani broth amended with tryptophan (5 mM) for 3 - 4 days. Cultures were centrifuged at 10,000 rpm for 20 min. Two ml of supernatant was mixed with two drops of orthophosphoric acid and 4 ml of salkowski reagent. Tubes were incubated at room temperature for 25 min. The intensity of pink color was recorded at 530 nm spectrophotometrically and the amount of IAA produced was extrapolated from the standard curve.

Screening for ACC deaminase activity

Screening for ACC deaminase activity of isolates was done based on their ability to use ACC as a sole nitrogen source. All the isolates were grown in 5 ml of trypticase soy broth medium incubated at 28 ^{0}C at 120 rpm for 24 h. The cells were harvested by centrifugation at 3000 *g* for 5 min and washed twice with sterile 0.1 M Tris-HCl (pH 7.5) and spot inoculated on petri plates containing modified Dworkin and Foster (DF) salts minimal medium (Dworkin and Foster, 1958) supplemented with 3 mM ACC as sole nitrogen source. Plates containing only DF salts minimal medium without ACC was taken as negative control and with $(NH_4)_2SO_4$ (0.2 % w/v) as positive control. The plates were incubated at 28 ^{0}C for 72 h. Growth of isolates on ACC supplemented plates was compared to negative and positive controls and concluded based on growth by utilizing ACC as nitrogen source.

Exo polysaccharide (EPS) production

Exo polysaccharide was extracted from 3 day old cultures grown in trypticase soy broth(15% PEG 6000 was added to trypticase soy broth for inducing stress). The culture was centrifuged at 20,000 rpm for 25 min and the supernatant was collected. Highly viscous cultures were diluted with 0.85 % KCl before centrifugation. The pellet was washed twice with 0.85 % KCl to completely extract EPS. The possible extraction of intracellular polysaccharides was ruled out by testing the presence of DNA in the supernatant by DPA reagent (Burton, 1956).

RESULTS AND DISCUSSION

Soil sampling and isolation of salt tolerant rhizobacteria

Saline rhizospheric soil samples were collected from Rangareddy, Mahabubnagar and Nalgonda districts of Telangana. Bacterial isolation was carried out using trypticase soy agar (TSA) medium with 2% NaCl. Based on the differences in the colony morphology and gram staining, individual bacterial colonies were selected. Single colonies were further streaked on the agar media and single pure colonies were selected and the bacterial isolates were maintained at -20 ^{0}C in 50% (w/v) glycerol.

Screening of the isolates for salinity tolerance

All the 80 isolates were screened for salinity tolerance at 2%, 5%, 10% and 15% NaCl concentration in Trypticase soy agar (TSA) medium. The presence of growth was recorded as positive (+). All the 40 isolates which showed tolerance upto 15% NaCl were further evaluated for potassium solubilization (Table 1).

Table 1. *In vitro* screening of salinity tolerant rhizobacteria for "K" solubilization

Isolate	NaCl tolerance				K solubilization (mm)
	2 % NaCl	5% NaCl	10 % NaCl	15 % NaCl	
Ref 1	+	+	+	+	-
PJ 18	+	+	+	+	14
PJ 19	+	+	+	+	16
PJ 20	+	+	+	+	15
PJ 41	+	+	+	+	14
PJ 47	+	+	+	+	19
PJ 48	+	+	+	+	17
PJ 69	+	+	+	+	4
PJ 70	+	+	+	+	15
PJ 71	+	+	+	+	6

+ = Growth present and -= No growth

Potassium solubilization

Among the 40 isolates, only 9 solubilized potassium on Alexandrov's agar medium with the range of 4 to 19 mm (Table 1). PJ 47 isolate showed maximum solubilization of 19 mm followed by PJ 48 (17 mm), PJ 19 (16 mm), PJ 20 and PJ 70 (15 mm), PJ 18 and PJ 41 (14 mm), PJ 71 (6 mm) and the minimum solubilization was recorded with PJ 69 (4 mm), the isolate PJ 47 showed 21.05 % increase over the least solubilizing isolate PJ 69. The reference strain (ref 1) used for salinity tolerance, showed tolerance upto 15 % NaCl but did not solubilize potassium.

Similar results were recordrd with Priyanka and Sindhu (2013) twenty bacterial strains among 137 cultures tested, showed significant potassium solubilization on mica powder supplemented plates in the range of 15 to 48 mg L^{-1}. In glucose amended medium broth, bacterial strains WPS73 and NNY43 caused 41.0 and 48.0 mg L^{-1} of K solubilization.

Cultural and morphological characterization

The isolates were further streaked onto different specific media like Jensen's agar (JA), King's B (KB) agar, yeast extract mannitol agar (YEMA) with 2 % NaCl and other non-specific bacteria were checked for their growth on nutrient agar (NA) with 2 % NaCl. All the isolates after growing on the specific media. Four were identified as *Bacillus* sp., 3 as *Pseudomonas* sp. and 2 as *Azotobacter* sp., according to the Bergey's manual of systemic bacteriology (Table 2).

Of the 9 isolates 4 were gram positive,rod shaped and showed formation of endospores. All are convex elevated,white colored, without pigmentation. Surface is smooth in 3 isolates and 1 is mucoid. Margin is irregular in all 4 isolates and these 4 were identified as *Bacillus* sp.

Three isolates were gram negative and didn't show endospores formation, all are rod shaped. Two isolates were dull white, 1 is yellow colored with convex elevation. Surface was smooth gummy in PJ 18, smooth shiny in remaining 2 isolates (PJ 19, PJ 20). All the three having regular margin but only two isolates showed pigmentation, PJ 18 showed brown and PJ 20 showed light green pigmentation (Table 2).

Two were gram negative, sporulation negative, white colored with convex elevation, smooth shiny surface and regular margin. All the 2 isolates were oval shaped with light brown and dark brown pigmentation and all the isolates produced cyst on Waksman No.77 N free agar medium.All the isolates were identified as *Azotobacter* sp. according to the Bergey's manual of systemic bacteriology (Table 2).

Table 2. Cultural and morphological characteristics of KSB isolates

Isolate name	Size	Shape	Color	Elevation	Surface	Margin	Pigmentation	Gram reaction	Sporulation
PJ 18	Medium	Rod	Dull white	Convex	Smooth gummy	Regular	Brown	Negative	Negative
PJ 19	Small	Rod	Yellow	Convex	Smooth shiny	Regular	-	Negative	Negative
PJ 20	Small	Rod	Dull white	Convex	Smooth shiny	Regular	Light green	Negative	Negative
PJ 41	Small	Rod	White	Convex	Smooth	Irregular	-	Positive	Positive
PJ 47	Small	Oval	White	Convex	Smooth shiny	Regular	Light brown	Negative	Negative
PJ 48	Small	Rod	White	Convex	Smooth	Irregular	-	Positive	Positive
PJ 69	Small	Rod	White	Convex	Smooth	Irregular	-	Positive	Positive
PJ 70	Small	Oval	White	Convex	Smooth shiny	Regular	Dark brown	Negative	Negative
PJ 71	Small	Rod	Dull white	Convex	Mucoid	Irregular	-	Positive	Positive

"-" Absent

Table 3 . Biochemical characterization of the KSB isolates

Isolate	Indole test	MR test	VP test	Citrate utilization	Catalase	Oxidase	Starch hydrolysis	Gelatin liquefaction	H_2S	Carbohydrate utilization			
										Lactose	Dextrose	Sucrose	Mannitol
PJ 18	-	+	-	+	+	+	+	+	+	+	+	-	+
PJ 19	-	+	+	-	+	+	+	+	+	+	-	-	-
PJ 20	-	-	+	+	+	+	-	+	+	-	+	-	-
PJ 41	-	+	-	+	+	+	+	+	-	+	+	-	-
PJ 47	-	+	-	-	+	+	-	-	-	-	+	-	+
PJ 48	-	+	-	+	+	+	+	+	+	+	+	-	-
PJ 69	-	-	-	+	+	+	+	+	-	-	+	+	-
PJ 70	-	+	-	-	+	+	-	-	-	-	-	+	+
PJ 71	-	+	-	-	+	+	+	+	-	+	+	-	+

+Positive **–**Negative **MR**-Methyl Red test **VP**-VogesPrausker's test **H_2S-** Hydrogen sulphide test

Similarly results were observed with Patil, 2014 who isolated and identified salt tolerant phosphate solubilizing bacterium from the soil as *Bacillus* sp. based on its morphological, cultural and biochemical characteristics. Damodaran *et al.* (2013) isolated 16 rhizobacteria through natural selection from saline soils and characterized them using morphological and biochemical parameters.

Biochemical characterization

After the study of cultural and morphological characteristics, the isolates were characterized with different biochemical tests *viz.,* IMVIC test, oxidase test, catalase test, carbohydrate utilization test, H_2S production, starch hydrolysis and gelatin liquefaction tests (Table 3).

Results presented in Table 3 reveal that all the isolates were negative for indole test. For the methyl red test 7 isolates were positive *i.e.,*PJ 18, PJ 19,PJ 41, PJ 47, PJ 48,PJ 70 and PJ 71. For Voges - prausker's test only 2 isolates were positive PJ 19 and PJ 20, citrate was utilized by only 5 isolateswhich include PJ 18, PJ 20, PJ 41, PJ 48 and PJ 69. All the isolates were positive for catalase and oxidase tests. Starch was hydrolysed by only 6 isolates PJ 18, PJ 19, PJ 41, PJ 48, PJ 69 and PJ 71. Gelatin was liquefied by 7 isolates except PJ 47 and PJ 70. H_2S was produced by only 4 isolates PJ 18, PJ 19,PJ 20 and PJ 48.For carbohydrate utilization test, the isolates were inoculated into four different carbohydrates *i.e.,* lactose, sucrose, dextrose, mannitol. In lactose agar 5 isolates utilized the carbohydrate lactose and showed positive for the test which include PJ 18, PJ 19,PJ 41,PJ 48 and PJ 71. Dextrose was utilized by 7 isolates except PJ 19 and PJ 70, sucrose was utilized by only 2 isolates which include PJ 69 & PJ 70. For mannitol utilization 4 isolates were positive for the test *i.e.,*PJ 18, PJ 47, PJ 70, PJ 71 and 5 isolates were negative for mannitol utilization (Table 3).

Similarly Patil *et al.* (2014) isolated 450 salt tolerant strains and all the isolates could grow at 12 per cent salt. Among which, 20 isolates were selected and subjected to morphological, biochemical examinations which exhibited the presence of great diversity. Jaymin *et al.* (2013) screened six isolates for NaCl at different concentration of which only two isolates showed tolerance upto 10% NaCl concentration. Biochemical and molecular (16 S r DNA sequencing) characterization revealed the strains to be *Exiguobacterium* sp. and *Serratia* sp. designated as GSD1 and GSD 2 strains.

Screening of KSB isolates for PGP attributes

Siderophore production

Out of nine isolates only 2 isolates produced siderophores (Table 4) and they showed strong production (+++) *i.e.,* PJ 47 and PJ 71 whereas seven isolates didn't produce siderophores *i.e.,* no production (-). Reference strain also showed weak siderophore production.

These results were in agreement with those of Sasirekha and Srividya (2016) who confirmed the siderophore producing ability of *P. aeruginosa* FP6. The maximum siderophore production was obtained in succinate medium (125 ìM) followed by King's B medium (105 ìM).

Hydrocyanic acid production

Hydrogen cyanide is a secondary metabolite produced by many antagonistic bacterial species from glycine. Four among 9 produced HCN, and all the 4 isolates produced weak HCN (+)*i.e.,*PJ 18, PJ 19,PJ 69&PJ 71 and five isolates didnot produce HCN *i.e.,* no HCN (-) (Table 4). Similarly all *Pseudomonas* strains produced optimum HCN production in 0 to 1 % NaCl but as the concentration increased from 1.25 to 2.25 % in medium, HCN production time varied from 24 h to approximately 48 h and above 1 % NaCl concentration (Deshwal and Kumar, 2013). Ahmadzadeh and Sharirifi-tehrani (2009) identified the production of HCN by six isolates out of the 41 selected Pseudomonads.

IAA production

Six isolates produced IAA among these strong (+++) production was shown by PJ 69 isolate, 5 isolates (PJ 41, PJ 47, PJ 48, PJ 70 and PJ 71) produced weak (+) amount of IAA and 3 did not produce IAA *i.e.,* no production (-). Reference strain also produced moderate amount of IAA (Table 4).

Similarly Ozdal *et al.* (2017) isolated 8 IAA producing bacteria from the rhizosphere of *Verbascum vulcanicum.* Among them, *Arthro bacteragilis* A17 gave maximum IAA production (75 mg/L). Nghia *et al.* (2017) isolated 213 IAA producing bacteria from fifteen soil samples within the salt affected areas of

rice crop. One out of ten efficient producers, the isolate ST2-1 was identified as the most promising strain and produced 33.13 mg.L^{-1} concentration of IAA after 8 days of incubation.

Screening for ACC deaminase activity

The isolates were evaluated for ACC deaminase activity and found its presence in four isolates among the 9 selected salinity tolerant KSB. They include PJ 18, PJ 47, PJ 70 and PJ 71. Reference strain also was negative for ACC deaminase activity (Table 4).

Similarly Mishra *et al.*(2017)isolated thirtyeight ACC deaminase producing PGPR which belong to 12 distinct genera and the isolates exhibited ACC deaminase activitiy ranging from 0.106–0.980 ìM α- ketobutyrateìg protein^{-1} h^{-1}.Kannika and Kedsukon (2012) selected *Bacillus licheniformis* B2r for its ability to utilize ACC as a sole nitrogen source under salinity stress and it also showed a high ACC deaminase activity at 0.6 M NaCl salinity.

Exo Polysaccharide production

Out of 9 strains, 6 showed positive for EPS production and 3 isolates were unable to produce EPS. Four isolates (PJ 18, PJ 19, PJ 20and PJ 70) produced moderate (++) amount of EPS, strong production (+++) was shown by the isolates (PJ 69 and PJ 71) and reference strain was negative for EPS (Table 4). The results were similar with Sandhya *et al.* (2015) Only 26 could tolerate maximum level of stress (-0.73 MPa) out of the 81 isolates, when monitored for EPS production. The strain GAP P45, showed the highest level of EPS production under water stress conditions, was identified as *Pseudomonas putida*on the basis of 16S rRNA sequence analysis.

Table 4. Screening of salinity tolerant PSB for PGP attributes, ACC deaminase and EPS production

Isolate name	Siderophore production	HCN production	IAA production	ACC deaminase*	EPS production
Ref 1	+	-	++	-	-
PJ 18	-	+	-	+	++
PJ 19	-	+	-	-	++
PJ 20	-	-	-	-	++
PJ 41	-	-	+	-	-
PJ 47	+++	-	+	+	-
PJ 48	-	-	+	-	-
PJ 69	-	+	+++	-	+++
PJ 70	-	-	+	+	++
PJ 71	++	+	+	+	+++

– No production, IAA -Indole Acetic Acid, HCN - Hydrogen Cyanide, + Weak production, ++ Moderate production, +++ Strong production, *ACC deaminase (+ presence and - absence), EPS -Exo polysaccharide

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Research Article
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PHENOTYPIC SCREENING OF THE BREEDING LINES OF MTU 1010 DERIVED THROUGH MARKER-ASSISTED PEDIGREE BREEDING FOR RESISTANCE AGAINST BACTERIAL BLIGHT AND BLAST

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ABSTRACT

In the present study, two major genes, viz., *Xa21* and *Pi54* conferring resistance against bacterial blight (BB) and blast, respectively were transferred into an Indian rice variety MTU1010. NILs of Akshyadhan (RP6132) possessing *Xa21* and *Pi54* was used to transfer the target traits into NIL of MTU1010 (RP5973-20-9-8-24-12-7), which is highly susceptible to both BB and blast diseases, but tolerant to low soil P. A marker-assisted pedigree breeding approach was adopted to combine low soil phosphorous tolerance, *Xa21* and *Pi54*. Plants at F_3 generation were observed to be highly resistant against bacterial blight and blast along with high yield, good agronomic attributes and low shattering of grains, which is a major problem in MTU1010. This work demonstrates the successful application of marker-assisted breeding for combining BB and blast genes (*Xa21* + *Pi54*) into a low P tolerant NIL of MTU1010 (RP 5973-20-9-8-24-12-7). All the 15 F_3 lines were also observed to be resistant to bacterial blight and blast through phenotypic screening and possessed grain yields and grain quality equivalent to or better than the MTU1010 NIL. Two breeding lines viz., LPK 30-18-16 and LPK 49-15-22 respectively, exhibited good performance with high level of resistance to all the three stresses viz., BB, blast and low soil P and possessed high yield under both normal soil P and low soil P with highly desirable long slender grain type like MTU1010.

Rice is one of the principal food crops for half of the world population of about 3.5 billion. India, ranks first in area (43.39 million hectares), second in production in the world (104.32 million tons) with an average productivity of 2.40 tones ha^{-1} (Agricultural Statistics at a Glance-2016; http://eands.dacnet.nic.in/latest_2006.htm). The rice production and productivity must be doubled by the year 2025, to meet the requirement of the increasing population (Hossain, 1996 and Mishra *et al.,* 2003). However, rice production is limited by many biotic stresses like bacterial blight (BB), blast, brown planthopper (BPH) etc. and abiotic stresses like salinity, drought, low soil phosphorus etc., Among the biotic stresses, two most devastating diseases in rice are blast which is caused by the fungus *Magnaporthe grisea* and bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* and can cause yield loss as high as 50% or more.

MTU1010, a short duration rice variety released in 2000 derived from the cross Krishnaveni/IR64, is extremely popular with farmers and has been planted for many years in a minimum of one million hectares due to its wider adaptability along with good level of tolerance to BPH, coupled with short duration and high yield. Despite these desirable features, MTU1010 is highly susceptible to bacterial blight (BB) and has moderate level of tolerance to blast disease, both of which cause significant yield loss in the variety in many states of India. To cope up with these two problems, development of resistant varieties and their cultivation is considered as the most effective, economical and environment friendly strategy.

Keeping in view of the above mentioned points, the present study was initiated to improve pre-breeding line of MTU1010 (RP5973-20-9-8-24-12-7) possessing *Pup1* QTL (a major QTL conferring excellent tolerance to low soil phosphorus conditions developed by ICAR-Indian Institute of Rice Research, Hyderabad; Anila *et al.*, 2014) for their resistance to BB and blast diseases. BB and blast resistant breeding line of another elite rice variety possessing high yield, long slender grain type, viz., Akshayadhan (named RP6132 and possessing *Xa21* and *Pi54* genes for resistance against BB and blast, respectively; Bhaskar Naik *et al.*, 2015) was used for marker-

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assisted breeding. The aim of the study was to develop improved versions of MTU1010 possessing *Xa21, Pi54* and *Pup1* along with higher level of yield and low grain shattering (a major problem in MTU1010).

MATERIAL AND METHODS

A NIL of Akshyadhan (RP6132) carrying BB and blast resistance genes of *Xa21* and *Pi54*, respectively was used to combine BB and blast resistance into a NIL of MTU1010 (RP5973-20-9-8-24-12-7), susceptible to both the diseases, but possessing the major QTL associated with low soil P tolerance. The NILs of RP6132 and RP5973-20-9-8-24-12-7 were developed by ICAR-Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, India The crossing programme was initiated during *Kharif,* 2016. F_2 plants homozygous for the target genes were selected with the help of gene-specific PCR based markers.

Screening of the breeding lines for their resistance against bacterial blight and blast:

Fifteen F_3 derived from the homozygous F_2s was screened for their resistance against bacterial blight and blast during *Rabi* 2018. TN1 and ISM were used as susceptible and resistant checks, respectively while screening for bacterial blight resistance. HR12 and Tetep were used as the susceptible and resistant checks, respectively during the screening for blast resistance. Screening for bacterial blight was carried out under glass house condition at ICAR-IIRR (Sundaram *et al*. 2008), while screening for blast resistance was done in the uniform blast nursery (UBN) at ICAR-IIRR (Rekha *et al*., 2008).

A virulent isolate of the bacterial blight pathogen, *Xanthomonas oryzae* pv. *Oryzae* (*Xoo*), DX020 was used for inoculation. Observations were recorded 15 days after inoculation by measuring the lesion length. The lines were categorized as resistant (lesion length <3 cm), moderately resistant (lesion length 3.1-5 cm) or susceptible (lesion length > 7 cm) as IRRI standard evaluation system (IRRI, 2013).

With respect to blast screening, the F_3 lines along with the parents, checks Tetep (resistant) and HR12 (susceptible) were evaluated for their reaction to blast disease using a local virulent isolate of the pathogen, SPI-40 under uniform blast nursery. The disease reaction was recorded 15 days after inoculation by adopting a 0-9 scale of IRRI standard evaluation system (IRRI, 2013). (Table:1)

Table 1: SES for evaluation of Blast of rice (IRRI, 2013)

Score	Description of symptom
0	No Lesions
1	Small brown specks of pinhead size without sporulating centre
2	Small roundish to slightly elongated, necrotic grey spots, about 1-2mm in diameter with a distinct brown margin and lesions are mostly found on the lower leaves
3	Lesion type is the same as in scale 2, but significant number of lesions are on the upper leaves
4	Typical sporulating blast lesions, 3mm or longer, infecting less than 2% of the leaf area
5	Typical blast lesions infecting 2-10% of the leaf area
6	Blast lesions infecting 11-25% leaf area
7	Blast lesions infecting 26-50% leaf area
8	Blast lesions infecting 51-75% leaf area

RESULTS

Phenotypic analysis of F_3 population for bacterial blight resistance: Fifteen F_3 derived from the homozygous F_2s were evaluated for bacterial blight resistance under glass house conditions at ICAR-IIRR (Figure 1). After 15 days of inoculation, scoring was done using IRRI SES scale. Out of the 15 F_3 lines evaluated against BB, four lines *viz*., LPK 28-19-15, LPK 38-5-18, LPK 49-1-21 and LPK 49-21-20 were observed to be highly resistant with a lesion length of 1 cm and other eleven lines were observed to be resistant with a lesion length of <3 cm.

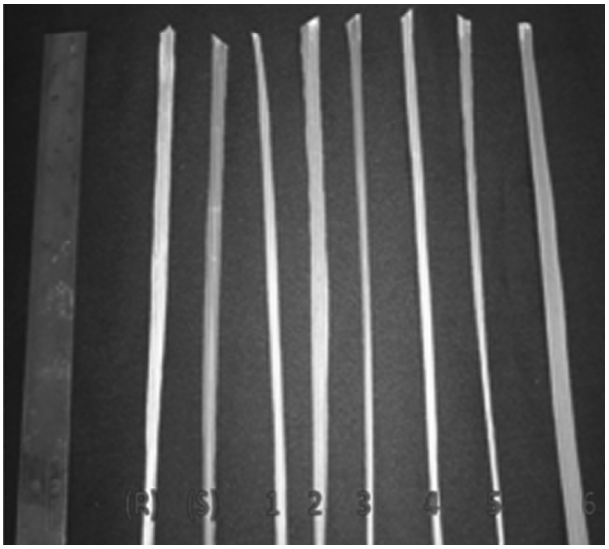
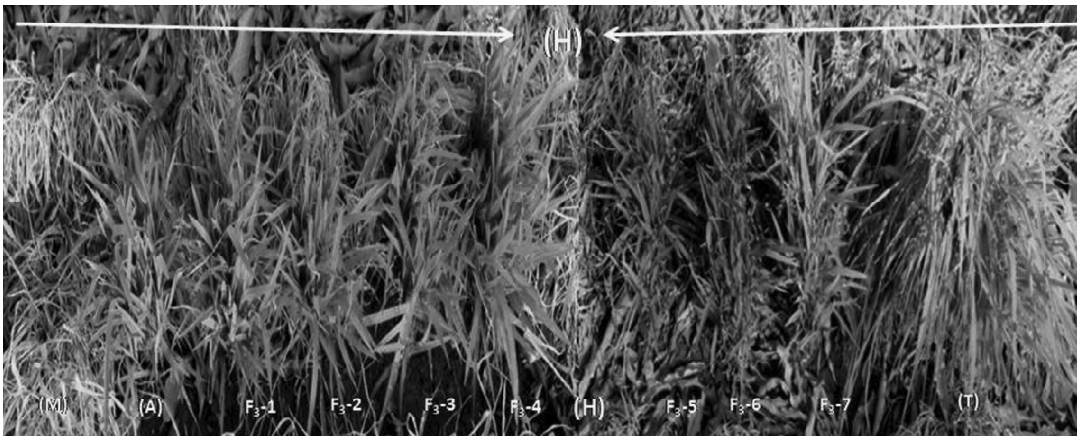


Figure1: Screening of F_3 breeding lines of MTU1010 (RP5973-20-9-8-24-12-7) x Akshyadhan (RP6132) for BB Resistance; (S):susceptible check- MTU 1010 (RP5973-20-9-8-24-12-7), (R) : resistant check : Akshyadhan (RP6132), F3-1 to F3-6: F_3 breeding lines of MTU1010 (RP5973-20-9-8-24-12-7) x Akshyadhan (RP6132). F3-1,2: LPK 28-19-15, F3-3,4: LPK 38-5-18, F3-5: LPK 49-1-21 and F3-6: LPK 49-21-20)

Phenotypic analysis of F_3 breeding lines for blast resistance:

Fifteen F_3 breeding lines derived from the homozygous F_2s were evaluated for blast resistance in uniform blast nursery at ICAR-IIRR (Figure 2). After 15 days of inoculation, scoring was done based on the IRRI SES score. Among the 15 breeding lines screened for blast resistance, 11 breeding lines were observed to be resistant with a score of 4 and four breeding lines viz., LPK 28-19-15, LPK 38-5-18, LPK 49-1-21 and LPK 49-21-20 with a score of 3. All the resistant breeding lines for blast were evaluated for agromorphological traits and advanced further to obtain promising lines possessing both blast and bacterial blight resistance genes.

Figure.2.Screening of F_3 breeding lines of MTU1010 (RP5973-20-9-8-24-12-7) x Akshyadhan (RP6132) for blast resistance in uniform blast nursery at IIRR. M: MTU1010 (RP5973-20-9-8-24-12-7), A: Akshyadhan (RP6132), H: HR12 and T:Tetep, F3-1 to F3-7: F_3 breeding lines of MTU1010 (RP5973-20-9-8-24-12-7) x Akshyadhan (RP6132). F3-1: LPK 28-19-15, F3-2: LPK 38-5-18, F3-4: LPK 49-1-21 and F3-7: LPK 49-21-20)



DISCUSSION

Acharya N.G Ranga Agricultural University (ANGRAU), Andhra Pradesh and Professor Jayashankar Telangana State Agricultural University (PJTSAU), Telangana state played an important role in Indian agriculture contributing towards the development and release of at least 10 mega rice varieties. Among them, MTU1010 (also known as Cottondora Sannalu) was released from ANGRAU is presently covering >12% of Indian rice acreage (Vanisree *et al.*, 2012), due to its wider adaptability along with good level of tolerance to BPH, coupled with short duration and high yield. Despite these desirable features, MTU1010 is highly susceptible to bacterial blight (BB) and has only moderate level of tolerance to blast disease, both of which cause significant yield losses in many states of India. Deployment of varieties with resistance genes is the most feasible approach to overcome these problems. The most effective approach to combat bacterial blight

is the use of resistant varieties (Khush *et al.,* 1989). Till date, at least 40 genes conferring host resistance against various strains of *Xoo* have been identified. *Xa21,* a major resistance gene, originally introgressed from *Oryza longistaminata* (Ronald *et al*., 1992; Song *et al*., 1995) was observed to confer resistance to most Indian isolates of the bacterial pathogen (Adhikari *et al*., 1999; Singh *et al*., 2001and Mishra *et al.,* 2013).

Another devastating disease, blast often results in significant yield loss to the tune of 70-80% during an epidemic. Hence, there is an urgent need to improve rice varieties by incorporating genes conferring resistance to bacterial blight and blast diseases. More than 100 loci conferring resistance to blast have been identified in rice (Sharma *et al*., 2012) and among them, *Pi-k*^{h}, which has been recently renamed as *Pi54* (Sharma *et al*., 2010), exhibited resistance to predominant races of the pathogen in India (Sharma *et al*., 2002). *Pi54* gene was originally identified from Tetep, a Vietnamese indica rice line and mapped on chromosome 11L with a set of tightly linked and functional markers (Sharma *et al*., 2005; Ramkumar *et al*., 2011). In order to sustain the yield levels of rice cultivars like MTU 1010, it is imperative to improve the variety for disease resistance. In the absence of effective chemicals or any other methods of control agents against BB and blast pathogens, resistance breeding is considered as the most economical and eco friendly strategy for management of the disease and achieving yield stability.

Pyramiding resistance genes is difficult to accomplish using conventional breeding strategy due to epistatic effects of genes controlling resistance, due to non-availability of screening facilities for multiple biotic stresses. Hence, marker assisted breeding demonstrated to be an efficient technique for precise transfer of one or few target genes into the genetic background of an elite variety or parental line. Earlier, Sundaram *et al*. (2008; 2009) and Hari *et al*. (2013), developed disease resistant versions of the varieties. In the present study following a similar approach, we have selected a single dominant gene, each conferring resistance against BB (i.e. *Xa21*) and blast (i.e. *Pi54*) for incorporation into an elite improved rice variety MTU1010 (RP RP5973-20-9-8-24-12-7), possessing low phosphorous tolerance QTL, *Pup1* through marker assisted pedigree breeding. Even though there were a few previous reports about breakdown of resistance conferred by a single BB resistance gene (Mew *et al*., 1992, and Khush *et al*., 1989) in rice, till date there was no report about large-scale breakdown of resistance conferred by either *Xa21* or *Pi54* from India or abroad. Further, as per a recent reports, NILs of Samba Mahsuri and Swarna possessing only *Pi54* displayed excellent resistance across multiple locations in India.

Through phenotypic and stringent marker assisted pedigree breeding, we were able to precisely transfer the target resistance genes, while ensuring recovery of almost all the good quality traits and yield of the MTU1010 NIL (i.e. RP5973-20-9-8-24-12-7) through careful phenotype based screening. A total of 15 improved F_3 breeding lines possessing two resistance genes along with *Pup1* were subjected for phenotypic screening for their bacterial blight and blast resistance. All these lines have shown good resistance to BB and blast diseases and among them, four breeding lines (*viz*., LPK 28-19-15, LPK 38-5-18, LPK 49-1-21 and LPK 49-21-20) have shown excellent resistance to BB (lesion length 1cm) and blast (score 3) (Figure1). Further, these lines showed a significantly lesser level of grain shattering as compared to MTU1010 (a major drawback of the mega-variety). The promising breeding lines, which were developed through this study are of tremendous potential due to their high level of resistance against BB and blast along with a desirable long-slender grain type and yield characters specific for MTU1010. These improved lines will be shortly evaluated in detail for their agromorphological and grain quality traits and promising lines will be later nominated for All India trials for their possible release and cultivation in areas endemic to bacterial blight and blast.

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Research Article
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EFFECT OF SOWING DATES ON GROWTH AND YIELD OF GREENGRAM (*Vigna radiata* L.) UNDER RAINFED SITUATION

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ABSTRACT

A field experiment was conducted during *kharif,* 2015 and 2016 at Agricultural Research Station, Madhira, Khammam district, Telangana to study the effect of optimum time of sowing in greengram. The experiment consisted of six dates of sowing (3rd wk of June, 1st wk of July, 3rd wk of July, 1st wk of August, 3rd wk of August and 1st wk of September) with three replications in randomized block design. Two years of study and pooled data at harvest showed that significantly higher plant height was recorded when greengram was sown in 3rd wk of June followed by 1st wk of July and higher drymatter was produced with 3rd wk of June followed by 1st wk of July sowing except in 2015 both were at par with each other. Sowing dates exerted significant effect on yield attributes (Clusters/plant, Pods/plant, Seeds/pod and Test weight) and seed yield of greengram were significantly higher in 3rd wk of June followed by 1st and 3rd wk of July compared to other dates of sowing in *kharif* season during two years and pooled.

Greengram (*Vigna radiata* L.) also known as mungbean or golden gram or moong or mungo has been grown since ancient times in India. The normal area, production and productivity of green gram during *Kharif* season has been 23.5 l.ha, 9.4 l.tonnes and 401 kg/ha respectively (Department of Agriculture, Cooperation and Farmers Welfare, Government of India, 2016). In Telangana, area, production and productivity of *Kharif* greengram is about 0.99 lha, 0.49 lakh million tones and 492 kg ha^{-1} respectively (Department of Agriculture, Telangana, 2016-17). It is good source of dietary protein for vast majority of people in India but still greengram is often grown in marginal lands with limited inputs exposing the crop to a number of abiotic stresses causing tremendous yield losses. Besides other constraints date of sowing is influenced by rainfall, temperature, photoperiod and sunshine hours which determine the phenological development of greengram at all growth stages. Water stress affects flower retention (Asaduzzaman *et al.,* 2008), pod development (Moradi *et al.,* 2008) as well as nitrogen accumulation and fixation (Thomas *et al.,* 2009). Sowing on time ensures that vegetative growth occurs during a period of optimum weather conditions also helps in grain filling and good grain quality. Delayed sowing results in the poor growth and yield. Under these circumstances, the standardisation of appropriate sowing time during *kharif* season seem to be important factor in achieving higher productivity under changed climatic conditions for greengram.

MATERIAL AND METHODS

The field experiment was conducted during the *Kharif* season of 2015 and 2016 in the Research Farm of Agricultural Research Station, Madhira. The soil of experimental site is alkaline condition (pH = 8.3), non-saline (Ec = 0.33), low in organic carbon (0.15) and available nitrogen (153 kg ha^{-1}), high in phosphorus (42 kg ha^{-1}) and potassium (538 kg/ha) contents. The mean maximum temperature during the crop season was 34.6°C and 31.3°C, while the mean minimum temperature was 27°C and 23.7°C in 2015 and 2016, respectively. Rainfall during the crop period was 654.1 mm (2015) and 751.1 mm (2016).

The experiment was laid in homogenous field in a randomized block design with six dates of sowing *viz.,* 3rd wk of June, 1st wk of July, 3rd wk of July, 1st wk of August, 3rd wk of August and 1st wk of September. Sufficient rain was received during both the years and there was no need of supplemental irrigation. Greengram seeds were sown at 25 kg ha^{-1} in rows spaced at 30 cm apart and 5 cm depth. After 20 days, thinning was done to maintain plant to plant distance of 10 cm. Nitrogen @ 20 kg ha^{-1} and phosphorus @ 40 kg ha^{-1} through DAP was applied at sowing. The data on plant height (cm), drymatter production (kg ha^{-1}), clusters/plant, pods/plant, seeds/pod and test weight (g) were recorded at harvest, which was tabulated and statistically analyzed. For comparing the means, the critical difference (C.D) was calculated at 5% level of significance.

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RESULTS AND DISCUSSION

Growth parameters, yield attributes and grain yield

Growth parameters (plant height and drymatter production) at harvest were significantly influenced by the dates of sowing during both the years of study. The tallest plant stature was recorded in greengram crop sown in 3rd wk of June (62.8 cm and 70 cm) followed by the plant height of crop sown in 1st wk of July (66.3 cm and 52.6 cm) during 2015 and 2016 respectively. Similar, results from pooled data was observed with significantly taller plants stature acquired by the crop sown in 3rd wk of June (66.4 cm) followed by 1st wk of July (59.5 cm). These results were supported by the findings of Gebologlu *et al.,* (1997), Ram and Dixit (2000) who reported significant effect of different sowing dates on plant height of mungbean sown on different dates and delay in sowing causes a substantial decrease in all the growth and development of mungbean. The drymatter production of greengram was significantly higher with 3rd wk of June sowing (1099 and 1045 kg ha^{-1}) and at par with 1st wk of July (1008 kg ha^{-1}) during 2015 and followed by 1st wk of July (691 kg ha^{-1}) in 2016 (Table 1). Study of pooled data reveals that significantly higher drymatter production was obtained by the crop was sown on 3rd wk of June (1072 kg ha^{-1}) followed by 1st wk of July (850 kg ha^{-1}). Algan, (2011) also noticed similar results. Late sown crop could not accumulate sufficient dry matter because of lesser vegetative and reproductive period. Timely sown crop accumulated more photo synthates. This may be due to favorable environmental conditions like temperature, rainfall, sunshine period etc., conducive for growth and development of the crop (Samant *et al.,*1999 and Miah *et al.,* 2009).

Table 1. Plant height and Drymatter production of green gram as influenced by sowing dates during *Kharif*, 2015 and 2016 (Pooled data)

Treatments	Plant height (cm)			Drymatter production (kg ha^{-1})		
	2015	2016	Pooled	2015	2016	Pooled
3rd wk of June	62.8	70.0	66.4	1,099	1045	1072
1st wk of July	66.3	52.6	59.5	1,008	691	850
3rd wk of July	35.1	38.6	36.9	755	614	685
1st wk of Aug	35.8	34.2	35.0	754	730	742
3rd wk of Aug	29.0	33.3	31.2	741	576	658
1st wk of Sep	31.5	32.1	31.8	731	806	769
SE(m)	1.1	1.4	1.1	50	59	36
CD	3.4	4.6	3.6	159	187	114

Among the yield attributes the more number of clusters/plant were recorded when the crop was sown in 3rd wk o June (10 and 5), 1st wk of July (10 and 6) and 3rd wk of July (8 and 5) which were at par with each other during two years of study. Pooled data of the crop resulted significantly more number of clusters/plant when it sown on 3rd wk of June (8), 1st wk of July (8) which were on par with each other and followed by 3rd wk of July (6). Early sowing of mungbean resulted in more number of clusters than delayed sowing. Significantly higher number of pods/ plant were produced in 3rd wk of June (31) followed by 1st wk (30) and 3rd wk of July (29) which were at par with each other during 2015 whereas in 2016 the crop produced more number of pods/plant in 3rd wk of June (22) followed by 1st wk of July (17) and 3rd wk of July (17). In pooled data over two years of study recorded significantly higher pods/plant in 3rd wk of June (26) followed by 1st wk (23) and 3rd wk of July (23) both were at par with each other (Table 2). Singh *et al.,* (2010) and Singh *et al.,* (2013) also reported similar result. High temperatures, precipitation and wind speed during the reproductive phase cause enormous bud and flower shedding resulted in lower pods per plant uunder delayed sowing of greengram.

Table 2. Yield parameters of green gram as influenced by sowing dates during *Kharif*, 2015 and 2016 (Pooled data)

Treatments	Clusters / Plant			Pods / Plant		
	2015	2016	Pooled	2015	2016	Pooled
3rd wk of June	10	5	8	31	22	26
1st wk of July	10	6	8	30	17	23
3rd wk of July	8	5	6	29	17	23
1st wk of Aug	7	3	5	24	13	19
3rd wk of Aug	6	2	4	24	13	18
1st wk of Sep	6	5	6	23	15	19
SE(m)	0.7	0.4	0.3	1	1	1
CD	2	1	1	5	2	2

It is evident from two years of study (Table 3) that significantly maximum number of seeds/pod were recorded under sowing of June 3rd wk (12,10) followed by July 1st wk (11,7) and July 3rd wk (10,7) which were at par with each other and significantly superior over other dates of sowings. An examination of pooled data indicates similar trend of significantly higher no of seeds/pod (11) was produced during June 3rd wk sowings compared to rest of the sowing dates. This might be due to decrease vegetative growth and increased reproductive growth through optimum planting and delayed in planting hampered number of seed per pod. Similar findings explained by Singh *et al.,* (2013). The test weight of the greengram has shown non significant effect by the different dates of sowing during two years of study and pooled data over years.

Significantly higher seed yield (kg ha^{-1}) was obtained by June 3rd wk sowing (1139, 854 and 996) followed by July 1st wk (1000, 832 and 916) and July 3rd wk (944, 820 and 882) which were at par with each other and higher than other dates of sowing during two years of study and pooled data respectively (Table 3). The findings are in conformity with results of Gebologlu *et al.,* (1997) and Borah (1997). These results revealed that early and optimum time of sowing of crop produced higher grain yield which resulted from the highest number of clusters/plant, pods/plant, seeds/pod and test weight. Grain yield decreased with delay in planting (Miah *et al.,* 2009, Singh *et al.,* 2010 and Singh *et al.,* 2013).

Table 3. Yield attributes and seed yield of green gram as influenced by sowing dates during *Kharif*, 2015 and 2016 (Pooled data)

Treatments	No. of seeds/pod			Test weight (g)			Seed yield (kg ha^{-1})		
	2015	2016	Pooled	2015	2016	Pooled	2015	2016	Pooled
3rd wk of June	12	10	11	32	39	36	1,139	854	996
1st wk of July	11	7	9	32	38	35	1,000	832	916
3rd wk of July	10	7	9	30	36	34	944	820	882
1st wk of Aug	8	5	6	29	36	33	639	622	630
3rd wk of Aug	8	5	6	29	35	32	694	546	620
1st wk of Sep	7	8	8	29	37	33	750	758	754
SE(m)	0.7	0.4	0.3	1.3	1.3	0.7	100	53	49
CD	2	1	1	NS	NS	NS	321	169	156

CONCLUSION

The present study revealed that sowing should be done early on 3rd wk of June to 3rd wk of July for obtaining high seed yield in mungbean.

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Research Article
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IN VITRO SCREENING OF HEAVY METAL TOLERANT PHOSPHATE SOLUBILIZING PLANT GROWTH PROMOTING ISOLATES

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ABSTRACT

The present study was conducted to isolate promising heavy metal tolerant bacteria from various industrial polluted areas of Hyderabad, India and to test for their plant growth promoting characteristics. Eight *Pseudomonas* strains isolated were tested for their maximum tolerance to heavy metals (Ni, Cd, Co, Pb) by supplementing their respective salts in different concentrations (0, 25, 50, 100, 150 and 200 mgl^{-1}) to King's B agar. Among all strains AfS-8,AfS-8, UpW-13 and SfW-14 were able to tolerate upto 100 mg l^{-1} concentration of heavy metals. All the isolates were also screened for various biochemical traits like mineral (P, K) solubilisation, HCN production, IAA production, Siderophore production, EPS production ACC deaminase activity.

Heavy metal pollution is currently a major environmental problem because metal ions persist in the environment due to their non-degradable nature. The toxicity and bioaccumulation tendency of heavy metals in the environment is a serious threat to the health of living organisms.

Bioremediation is a state-of-the-art technique used for heavy metal removal and/or recovery from polluted environments. The technique utilizes inherent biological mechanisms to eradicate hazardous contaminants using microorganisms and plants, or their products, to restore polluted environments to their original condition (Dixit *et al.,* 2015; Mani and Kumar, 2014). The cellular structure of a microorganism can trap heavy metal ions and subsequently sorb them onto the binding sites of the cell wall (Malik, 2004). This process is called biosorption or passive uptake, and is independent of the metabolic cycle. Among diverse soil microbes, the plant-growth-promoting bacteria (PGPB) produce plant-growth regulators, mineral solubilizers, phytohormones, and various secondary metabolites have been reported to expedite the plant-growth and development and soothe plants against various environmental stresses including metal stress. Moreover, they have shown excellent results in reducing metal toxicity by promoting plant-growth when used as inoculants.

The aim of this study was to characterize metal tolerant microbial strains having plant growth promotion traits isolated from heavy metal contaminated soil and to recommend them as potential bioinoculants.

MATERIAL AND METHODS

Study area and collection of samples

Heavy metal polluted soil and water samples were collected from three sewage irrigated agricultural sites (Afzalganj, Uppal, Student farm of College of Agriculture) in Hyderabad. Amount of heavy metals were analyzed in water and soil samples by atomic absorption spectrophotometer (AAS) given by Lindsay and Norvell (1978).

Metal Tolerance Test

Heavy metals tolerant bacteria were isolated on nutrient agar supplemented with various concentration (0, 50, 100, 150 and 200 mg l^{-1}) of $NiCl_2.6H_2O$, $CdSO_4$, $CoCl_2$ and $PbCl_2$. The agar amended with heavy metal salts was sterilized at 121°C for 15 min and allowed to cool to 40-45°C and transfer into petri plates. The pure cultures were streaked on heavy metal enriched medium and resistance was determined by the appearance of growth of bacteria after 3 to 4 days of incubation. The minimal inhibitory concentration (MIC) was determined as the lowest concentration of metal ion that completely inhibited growth.

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Isolation, screening and characterization of Heavy metal tolerant bacteria

Eleven strains of bacteria were isolated as metal tolerant. Qualitative characterization of all the eleven isolates were characterized by following standard protocols.

a) ***Determination of phosphate solubilization***: For estimation of phosphate solubilization all the isolates were inoculated on the Pikovskaya's agar medium. After 3 to 5 days of incubation at 28±2 °C, when bacteria solubilised the phosphate,a clear zone appeared around the spot inoculums. Halo zone around the growth was measured for the obtaining the phosphate solubilisation.

b) ***Potassium solubilization****:* Bacterial colonies exhibiting clear zone of potassium solubilization on Aleksandrov agar were selected as potassium solubilizers (Prajapati and Modi, 2012).

c) ***Production of Indole acetic acid:*** Indole acetic acid production by the isolates was quantitatively measured by the method given by Gordon and Weber (1951).

d) ***Production of HCN*** **:** All the isolates were tested for the HCN production with the help of methodology of Castric and Castric (1983).

e) ***Siderophore Production:*** Siderophore production was estimated qualitatively. By taking 0.5% of cell free culture supernatant and added to 0.5 mL of 0.2% aqueous Ferric chloride solution. Appearance of orange or reddish brown colour indicated the presence of Siderophore (Yeole and Dube, 2000).

f) ***EPS Extraction*** *:* The culture broth of all the isolates were centrifuged at 6000 rpm for 10 min to remove cells. Two volumes of cold ethanol (4°C) were added to the supernatant and the crude EPS precipitate was dried in a dessicator overnight (Rasulov *et al.*, 2013).

g) ***Screening for ACC deaminase activity*** *:* All the heavy metal tolerant isolates were grown in 5 mL of trypticase soy broth medium incubated at 28ºC at 120 rpm for 24 h. The cells were harvested by centrifugation at 3000 rpmfor 5 min washed twice with sterile 0.1 *M*Tris-HCl (pH 7.5) spot inoculated on petri plates containing modified Dworkin and Foster salts minimal medium (Dworkin and Foster, 1958) and supplemented with 3 mM ACC as sole nitrogen source. Plates containing only DF salts minimal medium without ACC was taken as negative control and with $(NH_4)_2SO_4$ (0.2% w/v) was taken as positive control. The plates were incubated at 28ºC for 72 h. Growth of isolates on ACC supplemented plates was compared to negative and positive controls and efficient strains were selected based on growth by utilizing ACC as nitrogen source.

RESULTS AND DISCUSSION

Initial Heavy metal concentration of various polluted and unpolluted samples

Among the four heavy metals estimated Pb was more ranging from 5.90- 14.58 mg kg^{-1} and exceeded the minimum permissible limit in soil and water (Table 1)

Isolation and cultural characterization of purified bacterial strains:

A total of 8 phosphate solubilising *Pseudomonas* bacterial isolates were isolated from soil and water by serial dilution and plate count method.These pure cultures of different bacterial isolates were preserved and used for further analysis.

Table 1. Heavy metal concentration of various polluted samples (mg Kg^{-1} or mg L^{-1})

Samples	Nickel (Ni)	Cadmium (Cd)	Cobalt (Co)	Lead (Pb)
Afzulganj soil	3.16	1.01	2.55	8.56
Uppal soil	2.98	1.98	1.59	12.61
Student farm soil	3.11	1.23	1.62	14.58
Afzulgang water	2.61	0.54	0.86	5.90
Uppal water	2.35	0.95	0.42	6.95
Student farm water	2.54	0.87	0.53	9.32

The isolates took about 48 h to establish their growth on King's B agar. All the isolates developed small to medium, smooth and shiny colonies and showed yellowish green to dull white, irregular, spreading, glistening, convex, opaque, viscid colonies. Under microscopic studies, these isolates exhibited gram negative nature with single, isolated, rod shaped cells without endospores. (Table 2)

Table 2. Morphological and cultural characteristics of *Bacillus* and *Rhizobium* isolates

Isolates	Gram reaction	Cell Shape	Colony characteristics
AfS-7	-ve	Rod	Yellowish green, Irregular, non-spreading, glistening, convex, opaque, viscid colony
AfS-8	-ve	Rod	Irregular, non-spreading, glistening, convex, opaque, viscid colony.
UpS-9	-ve	Rod	Yellowish green, Round, non-spreading, glistening, convex, opaque, viscid colony
SfS-10	-ve	Rod	Dull white, round, non-spreading, glistening, convex, opaque, viscid colony
SfS-11	-ve	Rod	Yellowish green, irregular, spreading, glistening, convex, opaque, viscid colony
AfW-12	-ve	Rod	Yellowish green, irregular, spreading, glistening, convex, opaque, viscid colony
UpW-13	-ve	Rod	Dull white Round, non-spreading, glistening, convex, opaque, viscid colony
SfW-14	-ve	Rod	Yellowish green, irregular, spreading, glistening, convex, opaque, viscid colony

Assessment of MIC against each heavy metal

Minimum inhibitory concentration (MIC) for each heavy metal was examined ranging from 25 to 200 mg L^{-1}(Table 3). All the isolates grew at 25 mg L^{-1} but among them only three bacterial strains (AfS-8, UpW-13 and SfW-14) were resistant to higher concentration of heavy metal salt (upto 100 mg/l). Among eight *Pseudomonas* isolates AfS-8 showed resistance to higher concentration (*ie* upto100 mg of Ni, Co; upto 150mg Cd and upto 200 mg of Pb salt L^{-1}).

***Invitro* screening of *Pseudomonas* isolates for their PGPR characterization**

All the isolates were purified and screened for various biochemical tests and shown in the Table 4. All the eight isolates were able to form clear zone of phosphate solubilisation on Piko vyskaya' sagar plate ranging from 3-14 mm. Among them, AfS-8 recorded the highest solubilisation zone (13.66 mm) followed by UpS-2 (13.00 mm).

All bacterial strains were found to be positive for IAA production ranging inconcentration from 2.55 to 23.54 µ gml^{-1} as determined by the development of pink color after reaction with Salkowski reagent. Strain AfS-8 showed highest IAAproduction (23.54 µ gmL^{-1}) with spectrophotometer readings.

Among the eight bacterial isolates, five isolates were grown on DF salts minimal medium plus either ACC or ammonium sulphate and were assayed for ACC deaminase (ACCd) activity by incubating the extract with ACC and observing the growth. Two isolates showed moderate (++) ACCd production (AfS-8 and UpS-9) and remaining three isolates showed weak (+) ACCd production.

The development of a mucoid aspect, indicated a possible EPS development by the strains; EPS production was shown by all the isolates and among them AfS-8 and UpS-9 showed more production.

Isolated bacterial strains were examined for hydrogen cyanide (HCN) production. Except one (AfW-12) all the isolates were positive for HCN and the change of filter paper from yellow to orange AfS-7 and AfS-8 were strong producers.

Siderophore production was shown by six isolates and among them AfS-8 and UpS-9 were strong producers (+++) showing intense brown colour at the end.

All the *Pseudomonas* isolates were positive for phosphate solubilisation. Singh *et al.* (2015) also isolated heavy metal (Pb and Cd) resistant bacterial strain and identified as *Pseudomonas putida* CG29 (N5) by 16S rDNA gene sequence analysis. Result of their study showed that isolates were positive for qualitative screening parameters *viz*, inorganic phosphate solubilization, nitrate reduction and nitrification. All the isolates showed various PGP characters also. Similarly, Meliani and Bensoltane (2016) screened heavy metal tolerant *Pseudomonas aeruginosa* (P8) isolated from waste water and three PGPR strains of *P. fluorescens* (P4, P9, P10) from different rhizospheres with PGPR traits like synthesis of amino-cyclopropane carboxylic acid (ACC) deaminase, indole-3-acetic acid (IAA) and PO_4solubilzation.

Bacterial EPS are implicated in a number of functions, such as adhesion to substratum, protection against anti-bacterial compounds and binding to organic molecules and in organic ions (Ma *et al.,* 2009). Kalita and Joshi (2017) reported *Pseudomonas* sp. W6 isolated from extreme habitat

Table 3. Heavy metal tolerance of *Bacillus* and *Rhizobium* spp.- MIC(mg L^{-1})

Isolates	Ni ($NiCl_2$. $6H_20$)	Cd ($CdSO_4$)	Co ($CoCl_2$)	Pb ($PbCl_2$)
AfS-7	100	100	50	100
AfS-8	100	150	100	200
UpS-9	100	50	100	150
SfS-10	50	100	50	100
SfS-11	50	50	50	100
AfW-12	100	50	25	100
UpW-13	100	100	100	150
SfW-14	100	100	100	150

Table 4. Multiple plant growth promoting activities of *Bacillus* and *Rhizobium* isolates from the Heavy metal polluted soil

Isolates	PO_4 Solubilisation (mm)	K Solubilisation	IAA Production (μg ml^{-1})	EPS Production	ACC deaminase	HCN Production	Sidero-phore Production
AfS-7	-	6.78	2.55	+	-	+++	++
AfS-8	13.66	14.67	23.54	+++	++	+++	+++
UpS-9	13.00	15.66	7.21	+++	++	++	+++
SfS-10	12.33	15.00	11.56	++	-	+	++
SfS-11	-	7.56	5.63	+	++	+	-
AfW-12	08.66	10.33	13.12	+	+	-	++
UpW-13	09.00	13.66	10.11	++	+	+	-
SfW-14	11.00	-	16.23	+	-	+	+

of hot water spring of North–East India showed for its Lead biosorption property. ICP-MS analysis revealed 65% and 61.2% removal of lead from the Synthetic Bangladesh Ground Water medium in batch culture and column study respectively which was higher when compared to biosorption capacity of *P. aeruginosa MTCC*2474, *P. alcaligenes* MJ7 from forest soil and *P. ficuserectae* PKRS11 from uranium rich soil. Exopolysaccharide released by the isolate which influenced biosorption revealed the presence of ligands assayed using microbial hydrophobicity and FTIR.

CONCLUSION

The study identified microbial strains tolerant to heavy metal are helpful for reduction of heavy metals in environment. Hence, we can use these isolates for minimization of toxicity and enhancement of plant growth by their PGPR activity under metal stress condition. This study is also helpful in identification of strains for bioremediation because of high level of MIC of isolates (indicating that the strains can tolerate high levels of heavy metals).

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COMPARISON OF PERFORMANCE BETWEEN THE TIME SERIES FORECASTING MODELS ARIMA AND ARIMAX IN FORECASTING THE RICE YIELD

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ABSTRACT

Rice being the staple food for more than 50% of the world population, it is very essential to forecast the production of rice so as to meet the need of the rapidly growing population. Forecasting is also essential for better planning and decision making. Many forecasting technics have evolved and it is the matter of prediction accuracy. In this study, two time series forecasting models, Autoregressive Integrated moving average (ARIMA) and Autoregressive integrated moving average with exogenous variables (ARIMAX) were compared to forecast the rice yield during both *kharif* and *rabi* seasons of Rajendranagar region of Telangana state. The exogenous variables used in the study are percentage of dead hearts and percentage of white ears which are the damage symptoms of rice yield due to yellow stem borer (*Scirpophaga incertulas*). To compare the effectiveness of these two models 26 years rice yield data of both *kharif* and *rabi* seasons pertaining to Rajendranagar region of Telangana state was used i.e., from 1990-2016. The results showed that Autoregressive integrated moving average model with exogenous variables (ARIMAX) performed reasonably well compared to the other model i.e., Autoregressive integrated moving average model (ARIMA) and hence can be applied for real life predictions and modeling problems.

Rice (*Oryza sativa l.*.) is the most important cereal crop of the world both in respect to area and production. It is the important staple food for more than 50% of the world population and provides 60-70 per cent body caloric intake to the consumers. Asia is the largest producer and consumer of rice in the entire world. The total Rice production in the world is 487.46 million metric tonnes as estimated by the United states Department of Agriculture in november 2017 (USDA). India ranks second in rice production in the world with the production of 165.3 million metric tonnes where as China ranks first with 210.1 million metric tonnes (Statistica, the statistical portal, 2017). India is a developing country with limited input requirements, soil-enriching properties and suitability for growing in areas, rice occupies a unique place in our agriculture system. Rice finds a prominent place in Indian meals and remains a primary source of nutrition for the majority of population of our country.

Telangana State is the newly formed state in India bifurcated from Andhra Pradesh during June 2nd 2014. It borders Maharashtra on North West, Karnataka on West and Rayalaseema region of Andhra Pradesh state on south. The region has an area of 114.84 lakh ha and a population of 352.87 lakhs as per 2011 census. It is 12th largest state in the country. It has 31 districts. The Krishna and Godavari rivers flow through the state from West to East. Agriculture in Telangana is dependent on rainfall and agricultural production depends upon the distribution of rainfall. The influence of South-West monsoon is predominant. South-West Monsoon (79%) is spread over the period from June to September, North-East Monsoon (14%) from October to December and the rest 7% rainfall is received during the winter and summer months. Telangana (31 districts) receives a normal rainfall of 906.6 mm in a year.

National rice self sufficiency has become a strategic issue and the ability to forecast the future enables the farm managers to take the most appropriate decision in anticipation of the future. Many forecasting techniques have evolved but accuracy of the time series forecasting is fundamental to many decision processes. One of the most popular and commonly used models for the forecasting research and practice is the autoregressive integrated moving average (ARIMA) model. In the ARIMA models, the desired forecasting is generally expressed as a linear combination. But real world time series are often full

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of nonlinearity and are influenced by many exogenuous variables. Hence, it is necessary to consider the effect of these exogenous variables which is taken care by the ARIMAX model.

MATERIAL AND METHODS

The main purpose of this study is to investigate the forecasting ability of the two forecasting models i.e., Autoregressive integrated moving average (ARIMA) and Autoregressive integrated moving average with exogenous variables (ARIMAX) and to determine which model performs better. For this study, the data pertaining to the rice yield for both *kharif* and *rabi* seasons pertaining to the AICRP-IIRR, Rajendranagar region of Telangana State has been taken for the past 27 years i.e., from 1990-2016 and the exogenous variables are damage caused by yellow stem borer (*Scirpophaga incertulas*) which is expressed in terms of percentage of dead hearts and percentage of white ears which are the damage symptoms caused by ysb. The above said secondary data has been taken from the annual progress reports of AICRP reports, ICAR- Indian Institute of Rice Research, Rajendranagar, Hyderabad.

I. Auto Regressive Integrated Moving Average (ARIMA):

ARIMA model has been one of the most popular approaches to forecasting. The ARIMA model is basically a data-oriented approach that is adapted from the structure of the data themselves. An auto-regressive integrated moving average (ARIMA) process combines three different processes namely an autoregressive (AR) function regressed on past values of the process, moving average (MA) function regressed on a purely random errors and an integrated (I) part to make the data series stationary by differencing. In an ARIMA model, the future value of a variable is supposed to be a linear combination of past values and past errors. Generally, a non seasonal ARIMA model, denoted as ARIMA (p,d,q), is expressed as

$$Y_t = F_0 + F_1 Y_{t-1} + F_2 Y_{t-2} + F_2 Y_{t-3} + \ldots + F_p Y_{t-p} + e_t - G_1 e_{t-1} - G_2 e_{t-2} - \ldots\ldots - G_q e_{t-q}$$

Where Y_{t-i} and e_t are the actual values and random error at time t respectively. F_i (i = 1,2,…p) and G_j (j = 1,2,…,q) are the model parameters. Here 'p' is the number of autoregressive terms, 'd' is the number of non seasonal differences and 'q' is the number of lagged forecast errors. Random errors e_t are assumed to be independently and identically distributed with mean zero and the common variance σ_e^2.

Basically, this method has three phases:

1) Model Identification

2) Parameter estimation and

3) Diagnostic Checking.

The auto-regressive integrated moving average (ARIMA) model deals with the non-stationary linear component. However, any significant nonlinear data set limit the ARIMA.

II. Autoregressive Integrated moving Average with Exogenous variables (ARIMAX) model:

Autoregressive integrated moving average with exogenous variable (ARIMAX) is the generalization of ARIMA (Autoregressive Integrated moving average) models. Simply an ARIMAX model is like a multiple regression model with one or more autoregressive terms and one or more moving average terms. This model is capable of incorporating an external input variable. Identifying a suitable ARIMA model for endogenous variable is the first step for building an ARIMAX model. Testing of stationarity of exogenous variables is the next step. Then transformed exogenous variable is added to the ARIMA model in the next step.

An ARIMA model is usually stated as ARIMA (p,d,q), where 'p' stands for the order of autoregressive

$$\Delta^d Y_t = \delta + \theta_1 \Delta^d Y_t - 1 + \theta_2 \Delta^d Y_{t-2} + \ldots\ldots\ldots + \theta_p Y_{t-p} + e_{t-1} \alpha_1 - \alpha_2 e_{t-2} \alpha_q e_{t-2}$$

process (Box and Jenkins, 1970). The general form of the ARIMA (p,d,q) can be written as

Where as Δ^d gives the differencing of order d i.e., $\Delta = y_t - y_{t-1}$ and "2 ="y_t-"y_{t-1}

$$\Delta^d Y_t = \delta + \beta X_t + \theta_1 \Delta^d Y_t - 1 + \theta_2 \Delta^d Y_{t-2} + \ldots\ldots\ldots + \theta_p Y_{t-p} + e_{t-1} \alpha_1 - \alpha_2 e_{t-2} \alpha_q e_{t-2}$$

In Arimax model we just add exogenous variable on the right hand side

Where X_t is the exogenous variable and β is the coefficient.

Forecasting Model

In the present study, the models have been developed on the basis of the secondary data of past 27 years for Rajendranagar region. The model is applicable for the areas having agro climatic conditions similar to Rajendranagar region. The data on the best check varieties have been used to nullify varietal differences. This is the standard practice while using the Time series data analysis.

Data pertaining to the rice yield, damage data caused by yellow stem borer which is in the form of percentage of dead hearts and percentage of white ears for the past 27 years is divided into two groups, they are training data and testing data. The training data is a set of data that will be used to perform analysis and determine the model. The testing data is a set of data that will be used to test the accuracy of the forecast results. Hence out of 27 years, 24 years data is taken as training data and 3 years data is taken for testing data. The data was analyzed using the software SPSS 20.

The ARIMA Model

Arima modeling process begins with Estimation ARIMA model. This estimation includes degree of autoregressive (p), differencing (d), and moving average (q) and seasonality factors. This data is tested for stationarity using sequence plots, because this autocorrelations approach zero exponentially after the second or third time lag. So degree of d for Arima models can be decided accordingly. After we find degree of d then we have to find degree of p and q to make ARIMA model. Prediction degree of p and q can be seen from the correlogram plot of autocorrelation function (ACF) and partial autocorrelation function (PACF).

The ARIMAX Model

Arimax model requires independent variables that acts as an additional variable. Independent variables that are used in this study are the percentage of dead hearts and percentage of white ears which are the damage symptoms caused by yellow stem borer (*Scirpophaga incertulas)* during different stages of rice crop.

Bayesian Information criteria (BIC):

It is a criterion for model selection among a finite set of models and is based on likelihood function. In case of model fitting it is possible to increase the likelihood by adding parameter, which may results in over fitting. BIC resolve this problem by introducing penalty term for the number of parameters in the model.

$$BIC = 2*\log(L) + m* \log(n)$$

Where,

L : Likelihood of the data with a certain model

n : Number of observations

m: Number of parameters in the model

Root Mean squared error (RMSE):

It is square root of mean squared error and is also known as standard error of estimate in regression analysis or the estimated white noise standard deviation in ARIMA analysis. It is expressed as:

$$RMSE = (1/T)\sqrt{\Sigma (P_t - A_t)^2}$$

Where,

P_t : Predicted value for time t

A_t : Actual value at time t and

T : Number of predictions.

RESULTS AND DISCUSSION

The data pertaining to the rice yield, dead hearts, white ears, damage due to bph (no.of bph/10 hills) during *kharif* and *rabi* seasons are given below of the AICRP-IIRR, Rajendranagar is given below.

Year	*Kharif* yield	Dh *kharif*	We *kharif*	*Rabi* yield	Dh *rabi*	We *rabi*
1990	4203	8.8	11.2	4404	7.2	10.8
1991	4360	9.6	11.2	4290	9.2	10.5
1992	4263	11.2	6.6	4365	10	6.4
1993	3157	10.6	6.7	4326	10.2	5.9
1994	3564	14	12.7	4521	14.2	12.9
1995	5413	12.9	12.8	4263	11.25	12.7
1996	4098	8.8	7.5	4658	10.3	6

Year	*Kharif* yield	Dh *kharif*	We *kharif*	*Rabi* yield	Dh *rabi*	We *rabi*
1997	4523	4.1	4.2	3993	7.5	6.8
1998	3015	10.5	10.2	6318	15.8	8.2
1999	4184	4.5	6.3	5362	13.33	8.8
2000	4316	6.8	12.36	6218	11.2	8.6
2001	2870	4.2	2.9	3752	6.3	16.45
2002	3281	4.2	12.9	3854	15.8	16.2
2003	4189	3.1	11.38	5623	17.7	9.3
2004	4895	6.2	3.3	3093	6.4	14.6
2005	4088	4	3.3	3842	10.4	12.4
2006	3472	13.1	16.2	6581	10.57	6.1

Year	*Kharif* yield	Dh *kharif*	We *kharif*	*Rabi* yield	Dh *rabi*	We *rabi*
2007	3425	4.4	12.98	5389	25.2	11.9
2008	4515	1.5	3.6	5698	20.7	10
2009	5467	3.8	0.9	6966	15.3	13.1
2010	4562	8.5	6.4	4620	6	14.7
2011	3310	16.3	17.6	7069	11.9	11.8
2012	1613	11	18	3565	7.1	30.6
2013	4485	5.6	10.8	3968	8.2	11.06
2014	3565	11	7	1584	6	3.6
2015	3998	6	3.6	1684	7.7	12.3
2016	5495	7.7	12.3	5485	17.7	9.3

ANOVA Table with Regression coefficients

ANOVA	*df*	*SS*	*MS*	*F*	*Significance F*
Regression	3	3665039.7	1221679.9	1.793188	0.176588
Residual	23	15669652	681289.22		
Total	26	19334692			

Table for regression coefficients

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95%	Upper 95%
Intercept	4960.490404	466.0077666	10.644652	2.32E-10	3996.48	5924.501	3996.48	5924.501
X Variable 1	-12.90882579	51.89301605	-0.248758	0.805755	-120.258	94.44006	-120.258	94.44006
X Variable 2	-65.74249372	41.96323186	-1.566669	0.130848	-152.55	21.06506	-152.55	21.06506
X Variable 3	-0.900735068	0.860518804	-1.046735	0.306098	-2.68085	0.879384	-2.68085	0.879384

ARIMA model for the yield of rice during *Kharif* and *Rabi* seasons

Initially the data pertaining to the yield during *kharif* season for the past 26 years i.e., from 1990-2016 was taken and a sequence plot, autocorrelation function and partial autocorrelation function was verified to test for the stationarity of the time series data.

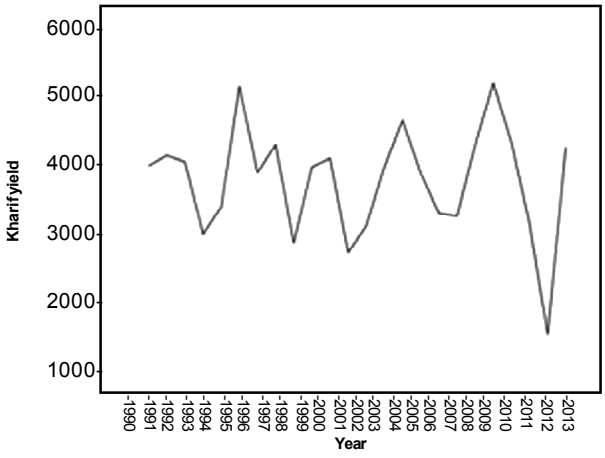

Fig.1. Showing the sequence plot plotted for year versus *kharif* yield

The sequence plot is mostly stationary with a very few fluctuations. Hence we go for acfs and pacfs.

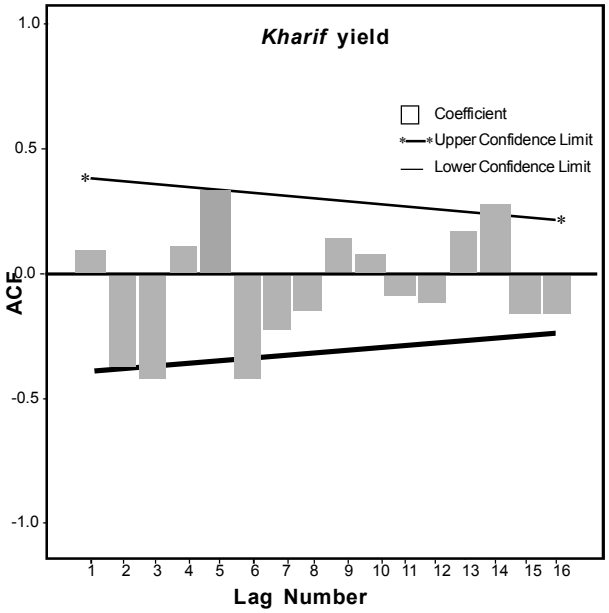

Fig. 2. Showing the autocorrelation function of *kharif* yield

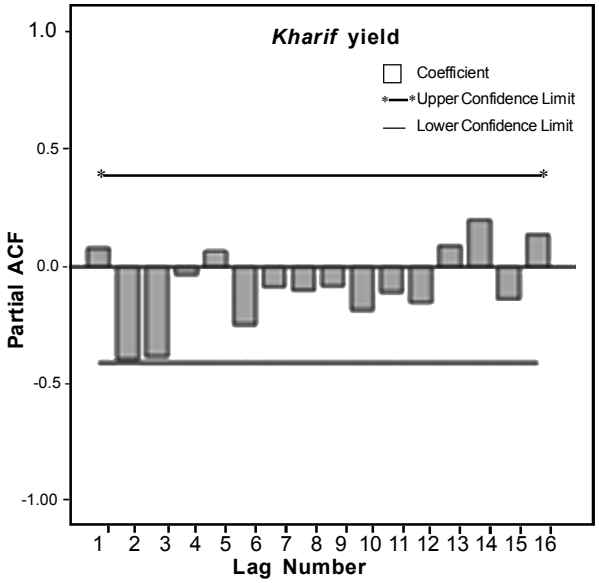

Fig. 3. Showing the partial autocorrelation function of *kharif* yield

The acfs and pacfs showed significance in lags 2, 3, 5 and 15 and the decay was not towards zero so we go for differencing and the sequence plot, acf and pacfs are given below which show that the series has become stationary on differencing.

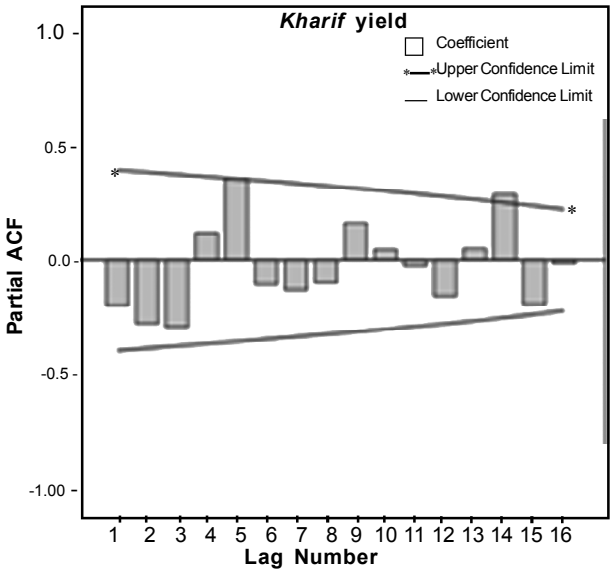

Fig.4. Showing the autocorrelation of *Kharif* yield after differencing

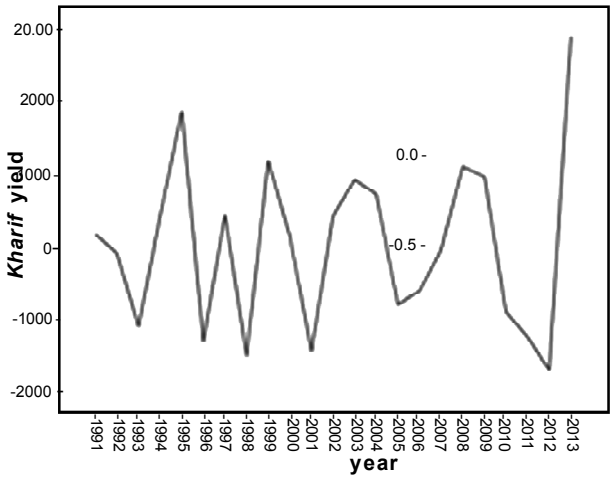

Fig.5. Showing the sequence plot of *kharif* yield after differencing

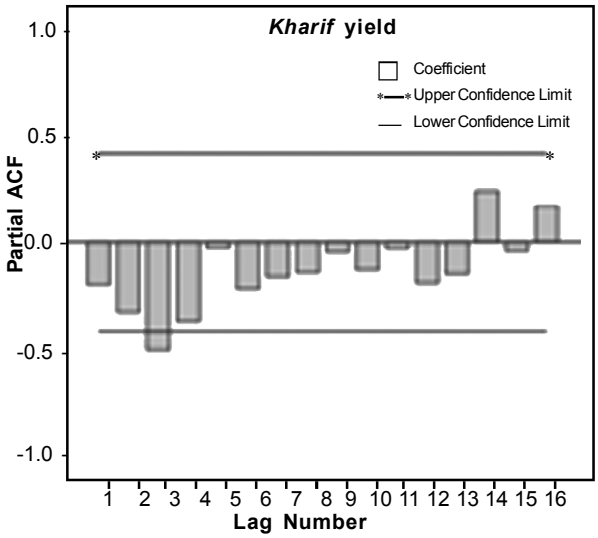

Fig.6. Showing the partial autocorrelation function of *kharif* yield after differencing the series

After deciding the order of differencing different iterations were performed and the best model was fit based on the least value of BIC and rmse. The different iterations were

Table 1. Showing the different ARIMA models fit for *kharif* yield

S.No.	Model	Normalized BIC	RMSE
1.	(0,1,0)	14.28	1179.96
2.	(1,1,0)	14.40	1173.92
3.	(0,1,1)	13.94	931.918
4.	(1,1,1)	14.58	1177.23
5.	(2,1,0)	15.38	1207.41
6.	(1,0,1)	14.01	978.36
7.	(2,0,1)	15.14	1156.32

And out of all the iterations the model (0,1,1) had the least bic and rmse values so it was chosen to be the best fit model.

For yield during *rabi* season also same procedure was followed and the stationarity of the data was verified using sequence plots, autocorrelation function and partial autocorrelation function.

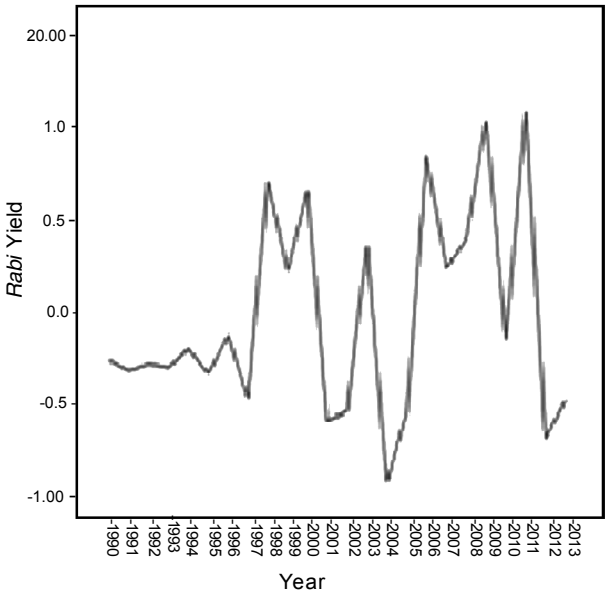

Fig.7. Showing the sequence plot of *rabi* yield

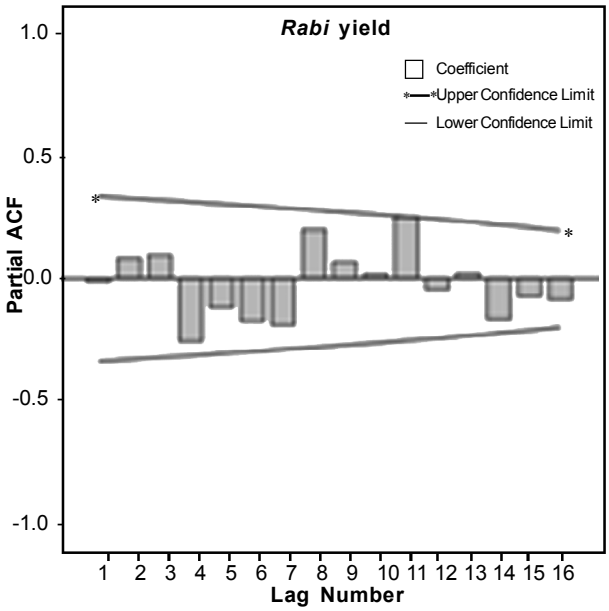

Fig. 8. Showing the Autocorrelation function of *rabi* yield

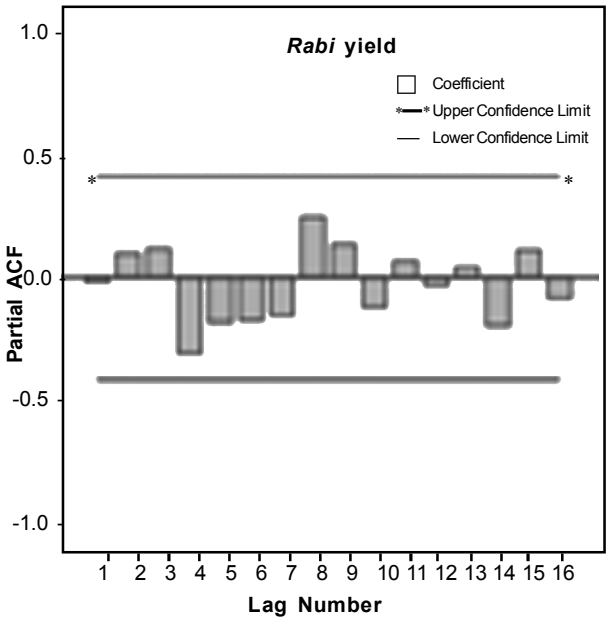

Fig.9. Showing the partial autocorrelation function of the *rabi* yield

The sequence plot showed fluctuations but the curve is showing a constant mean which means that the data is stationary. The ACFs and PACFs also showed significance at lag 3, lag 5 and both the series gradually decayed towards zero which showed that the data is stationary and no differencing is needed. So different iterations were tried and different arima models were fit for the *rabi* yield and the model with least value of BIC and rmse was chosen as the best model.

Table 2. Showing different ARIMA models fit for *rabi* yield

S.No.	Model	Normalized BIC	RMSE
1.	(0,1,0)	14.93	1630.92
2.	(1,1,0)	14.71	1368.35
3.	(0,1,1)	14.43	1188.94
4.	(1,1,1)	14.61	1214.18
5.	(2,1,0)	15.03	1223.22
6.	(1,0,1)	14.51	1161.01
7.	(0,0,1)	14.36	1154.33
8.	(1,0,0)	14.56	1176.18

Hence the model (0,0,1) had the least value of BIC and rmse hence (0,0,1) is the model of best fit.

ARIMAX model for the rice yield during *Kharif* and *Rabi* seasons

Yellow stem borer (*Scirpophaga incertulas*) is one of the major pests caused damage to the rice yield. It damages the crop leaving two symptoms and dead hearts and white ears. Hence the percentages of dead hearts and percentages of white ears are taken as the exogenous variables in forming the arimax model. To test for stationarity of the time series data during *kharif* season sequence plots, Autocorrelation functions and partial autocorrelation functions were examined individually for percent dead hearts and percent white ears.

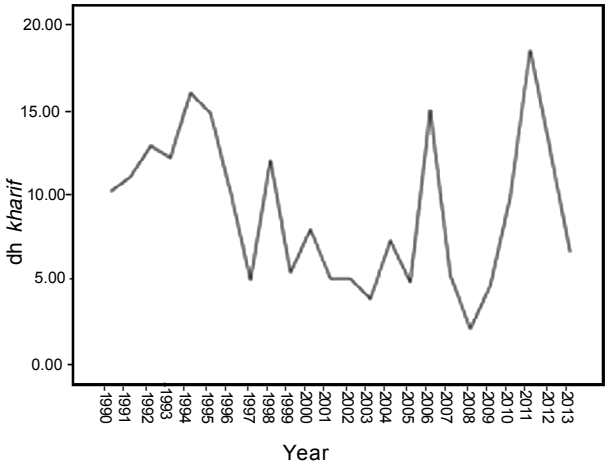

Fig.10 Showing the sequence plot for dead hearts percent during *Kharif* season

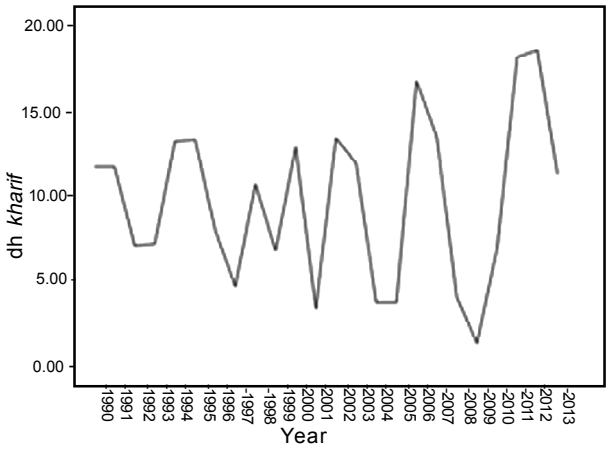

Fig.11 showing the sequence plot for white ears percent during *kharif*

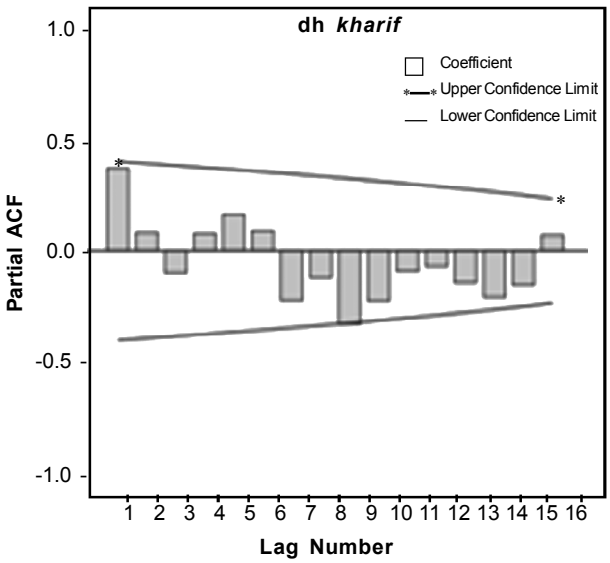

Fig.12 Showing the autocorrelation function of Dead hearts during *Kharif*

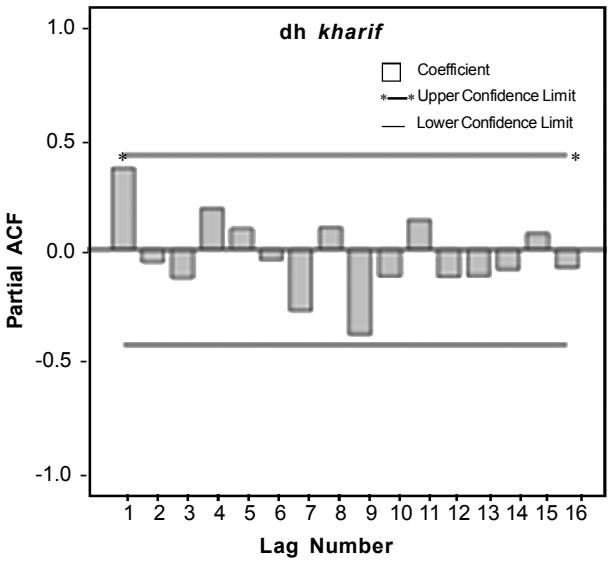

Fig.13. showing the partial autocorrelation function of dead hearts during *kharif*

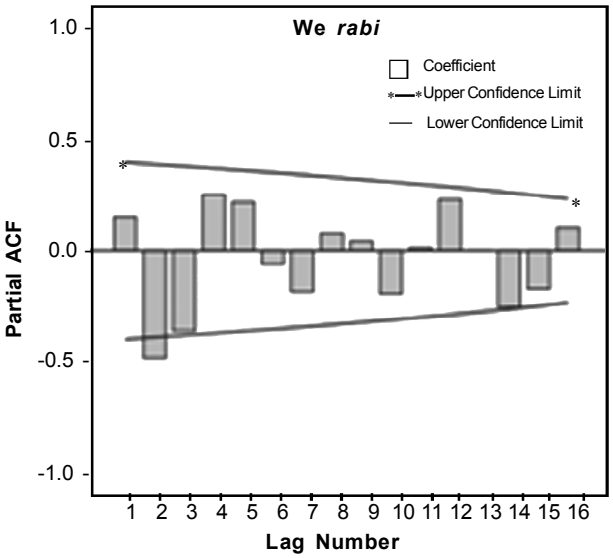

Fig.14 Showing the acf of white ears during kharif

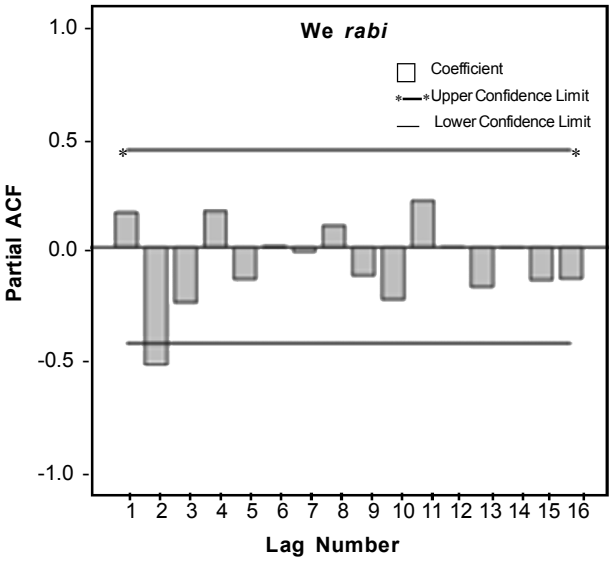

Fig.15.Showing the pacf of white ears during *kharif*

On examining the sequence plots, autocorrelation functions and partial autocorrelation functions we notice that the sequence plots exhibit stationarity in mean, acf and pacf of dead hearts show that the first lag and ninth lag are significant but all the lags lie within the limits which show that the acf and pacf of dead hearts also exhibit stationarity. When we examine acf and pacf of white ears, the second lag is significant and all other lags are insignificant. Hence being inconclusive about differencing we test all the iterations possible with and without differencing to fit the best fit arimax model.

Table 3. Showing different ARIMAX models fit for *kharif* season

S.No.	Model	Normalized BIC	RMSE
1.	(1,0,0)	13.88	795.59
2.	(0,0,1)	13.69	723.36
3.	(1,0,1)	13.78	708.40
4.	(0,1,1)	14.27	955.95
5.	(2,0,1)	15.04	1073.24
6.	(1,1,1)	14.53	978.21
7.	(1,1,0)	14.37	969.56

Out of all the iterations the model (0,0,1) has the least value of BIC hence it is the model of best fit.

Fitting ARIMAX model for *rabi* yield

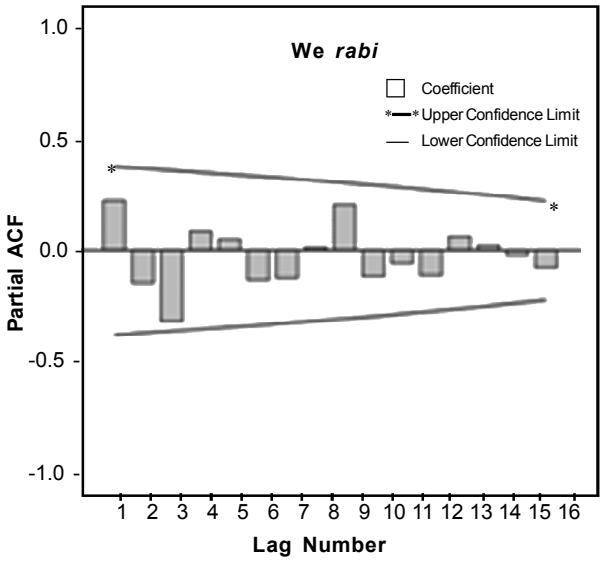

Fig.16. Showing the autocorrelation function Percent dead hearts during *rabi*

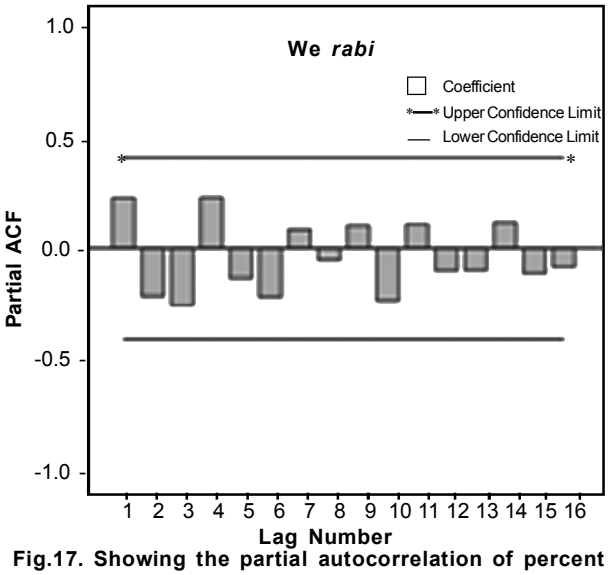

Fig.17. Showing the partial autocorrelation of percent dead hearts during *rabi*

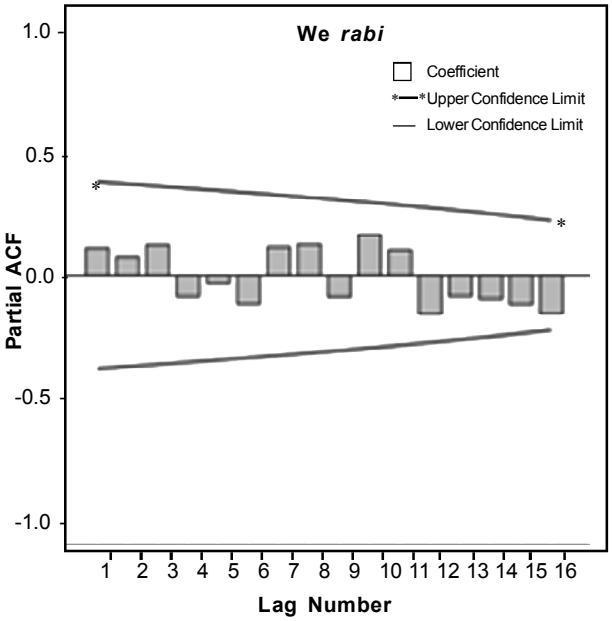

Fig.18. Showing acf of white ears during *rabi*

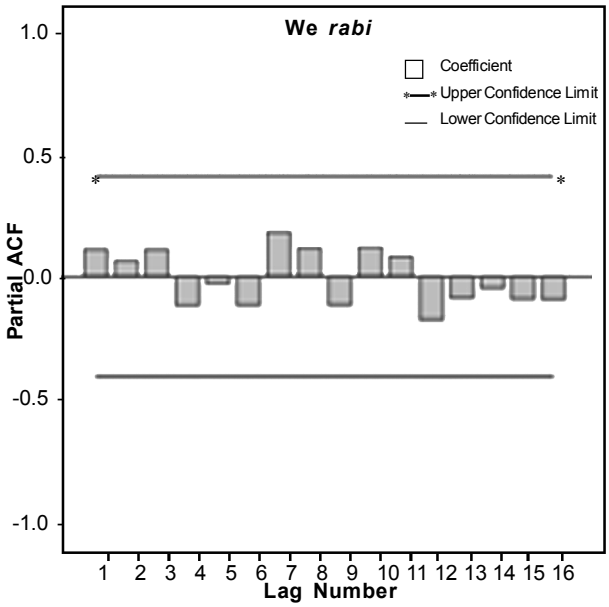

Fig.19. Showing pacf of white ears during *rabi*

The acfs and pacfs for dead hearts and white ears were examined separately and concluded that as non of the lags are significant the series is stationary and there is no need for differencing of the time series values. Different iterations were taken for arimax models to get the best fit model for the rabi season.

Table 4. Showing different ARIMAX models fit during *rabi* season

S.No.	Model	Normalized BIC	RMSE
1.	(1,0,0)	14.293	974.37
2.	(0,0,1)	14.298	976.62
3.	(1,0,1)	14.466	994.31
4.	(0,1,1)	14.166	907.31
5.	(1,1,0)	14.563	1106.22
6.	(1,1,1)	14.226	873.169
7.	(2,0,1)	14.781	989.25

Out of all the iterations the model (0,1,1) has the least value of BIC hence the model (0,1,1) is concluded to be the best fit arimax model.

Table 5. Showing comparative study of ARIMA and ARIMAX models:

S.No.	Season	ARIMA			ARIMAX		
		Model	BIC	RMSE	Model	BIC	RMSE
1.	*Kharif*	(0,1,1)	13.94	931.91	(0,0,1)	13.69	723.36
2.	*Rabi*	(0,0,1)	14.36	1154.33	(0,1,1)	14.16	907.31

Conclusion:. It is seen from the comparison of BIC and RMSE values that these values are smaller in ARIMAX than in ARIMA. Therefore ARIMAX model have better performance than the ARIMA model. Hence it can be concluded that the MSE will only be reduced on inclusion of those exogenous variables which are responsible for explaining the variability in the variable of interest. Hence the results in ARIMAX models are better than ARIMA.

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IN VITRO PROTEIN DIGESTIBILITY OF MILLET MEAL PREPARATIONS

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ABSTRACT

Six different millet products have been developed and standardized to serve as most common lunch dishes namely millet rice, *kichidi* and *roti* using traditional local recipe adopted for cereals. The overall mean *in vitro* protein digestibility (IVPD) of millet *kichidi* from 6 millets was 66.93 ± 3.78%, and it was significantly high (P<0.01), compared to the mean IVPD of millet *roti,* millet rice and raw millet in the decreasing order. Mean IVPD of millet *roti*, millet *rice*, and raw millet from the 6 millets showed no significant difference indicating that, dry roasting as in *roti* and wet cooking as in millet *rice* did not have much effect on digestibility of millets. Boiling as rice improved IVPD of finger millet and sorghum, but not in other millets. Dry roasting in *roti* improved IVPD in dehulled millets. Addition of legumes in *kichidi* improved protein quantity and protein digestibility *in vitro.* The difference of IVPD in millets varied from 3 to 10%.

Millet protein was superior to that of wheat or corn in terms of essential amino acids, and contained less than half the amount of the essential amino acid lysine that is found in high quality protein sources such as meat. In different grain varieties, higher the protein content, lower was the lysine content in the protein. In addition millet has antioxidant activity (Choi *et al*., 2007), anti-carcinogenic effects (Ha *et al.,* 1998), DPPH radical scavenging activity and anti-mutagenic affects (Kwak *et al*., 2004) and can reduce the risk of cardio-vascular disease (Cho *et al.,* 2000). Importance of phenolics in millet by protecting against stress was also reported (Dicko *et al*., 2006). Despite its superior nutritional quality it has received less attention compared to the major cereals. Gradually millet is gaining importance not only in our country, but also in the North American and European countries due to its gluten-free and hypoglycemic property.

Cooking generally reduced the *in vitro* protein digestibility of the millet significantly (p<0.05) (Amir *et al.,* 2009). It was relatively high in leucine and methionine. The starch in some foxtail millet varieties contained 100% amylopectin, and the starches contained in foxtail, proso and barnyard millets were more digestible than maize starch.

Studies have indicated that among the millets Barnyard millet recorded highest protein content (15.07%) and lowest was in Proso millet (8.5%). The average protein content in little, Kodo and Foxtail millet were reported to be about 9.5, 8.8 and 11.07 per cent respectively, with varietal differences within species as reported by several investigators (Malleshi and Desikachar, 1986; Monteiro *et al*., 1988; Hadimani and Malleshi, 1993; Kumar and Parameshwaran, 1998 and Veena *et al*., 2005). The few studies that have investigated the influence of protein content on ad libium energy intake have found that consuming high-protein foods decreased energy intake within a single meal.(Porrini *et al*., 1997)

Digestibility may be used as an indicator of protein availability. It is essentially a measure of the susceptibility of protein to proteolysis. A protein with high digestibility is potentially of a better nutritional value than one of low digestibility, because it would provide more amino acids for absorption on proteolysis. Their function in nutrition is to supply adequate amounts of needed amino acids. The protein nutritional quality of food depends on content, digestion, absorption and utilization of amino acids. However, there is growing emphasis for improvement of protein quality and quantity in cereal crops. Attempts have been made to fortify these cereals with legumes (FAO, 1995).

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The digestibility of cooked sorghum gruel was lower than that of cooked gruels made with wheat, mazie, rice and millet (Ramachandra *et al.,* 1977). The pepsin protein digestibility of decorticated, heat extruded sorghum was higher than whole sorghum gruels. The IVPD of cereals and millets, reported by Rajyalakshmi and Geervani (1990) indicated a reduction of 9 to 24 percent due to boiling.

Begum *et al.* (2003) carried out experiments on nutritional enhancement of common convenience foods such as papads by substituting conventional grains with nutritious millets. Acceptable papads were formulated using Finger millet (60%), sago (20%), black gram (20%) and spices. Calcium content was observed to be exceptionally higher in papads with Finger millet (156 g/100 g) as compared to traditional papads (82 mg/100 g).

Milling and heat treatment during *chapati* (an unleavened bread) making lowered polyphenols and phytic acid and improved the protein digestibility and starch digestibility to a significant extent (Chowdhury *et al*., 1997). *Roti* and *kichidi* were the most consumed meals in India because of their dense nutrient content (Rao and Deosthale,1983).

Higher IVPD was observed in little millet grain (59.92%) compared to finger millet with low IVPD of 45.32%. This was due to the established fact that, as the proportion of pericarp and germ material become less the IVPD improves (Duodu *et al*., 2002; Chibber *et al*., 1980).

Amir *et al.* (2009) reported the *in vitro* protein digestibility of Sudanese and Indian cultivars of sorghum as 49.25 and 55.85% for uncooked sample, while *in vitro* protein digestibility was 26.11 and 33.11% for cooked samples.

Hamaker *et al*. (1987) found that sorghum cooked in the presence of 2–Mercaptoethanol or other reducing agents had a significant increase (25%) in protein digestibility compared to sorghum cooked in water alone. It was proposed that the reducing agents open up the protein matrix through the cleavage of disulphide bonds allowing the digestive enzymes more accessible to the protein bodies.

MATERIAL AND METHODS

The selected six millets, namely pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), proso millet (*Penicum miliaceum*), foxtail millet (*Setaria italica*), little millet (*Panicum sumatrense*) and sorghum (*Sorghum bicolor*) were procured from Grameenmall Foundation, ALEAP Industrial Estate, Gajularamaram, Hyderabad, Telangana. Pearl millet, finger millet and sorghum grains were obtained in whole form as they are edible with hull, while foxtail millet, little millet and proso millet were obtained in dehulled form. Pearl millet, finger millet and sorghum were crushed to coarse semolina to enable the grain to cook like meal, while dehulled foxtail millet, little millet and proso millet were used as such. All millets were also milled to fine flour to suit preparation of millet *roti.*

Three most common Indian daily meal dishes namely rice, *kichidi* and *roti* were developed with each of the six millets using the traditional basic recipes. The recipes with millets were standardized, with variations in proportion of water used and soaking time and the products were subjected to acceptability studies and highly acceptable recipe was taken for preparation of final millet rice, millet *kichidi* and millet *roti*.

Preparation of millet rice, *kichidi* and *roti*

Millet rice was prepared by soaking the millet grain or coarse semolina for half an hour with 1:3 millet: water ratio and cooked to a soft rice consistency. Millet *kichidi* was made by soaking the millet grain or coarse semolina + green gram dhal in 4:1 proportion for half an hour with 1:4, millet:water ratio and cooked to a soft meal consistency and then seasoned with pepper, chillies, ginger, curry leaves fried in oil. Millet *roti* was prepared by mixing millet flour with just sufficient warm water to make soft dough and then divided the dough into big size lime and flattened to *roti* on a wooden board with roller pin and aid of dry flour and fried on a hot pan with or without oil.

The products were subjected to analysis of protein and *in vitro* protein digestibility on dry weight basis.

Analysis of protein

Dehydrated and finely powdered millet *meal, kichidi* and *roti* along with raw millet flours of each of the six millets were initially analyzed for protein, so as to take sample containing 6.75 ± 0.1mg of nitrogen for estimation of protein digestibility.

The crude protein content of the sample was estimated according to the Micro Kjeldhal Method (AOAC, 2005) and calculated as percent nitrogen of product and multiplied with 6.25 to obtain the protein content.

For estimation of protein, 0.5g of each sample was weighed in to the digestion tube and five grams of digestion mixture (98g of potassium sulphate + 2g copper sulphate) plus 10.00 ml of concentrated H_2SO_4 were carefully added and the samples were placed in the digestion unit for 1½ hr at 375°C.

In a 100 ml conical flask, 40.00 ml of 4% boric acid was added along with few drops of mixed indicator containing (1ml of 0.2% bromocresol green + 3 ml of 0.2% methyl red). Distillation was done for10 minutes in the Kjeldhal distillation apparatus adding 15.00 ml of 40% NaOH and steaming for 10 seconds. The contents collected in conical flask were blue in color after distillation was completed. Titration was done with standard 0.1N HCl till the contents of the flask turned to pink color. A blank was run simultaneously.

$$\% \text{ nitrogen} = \frac{(S - B) \times N \text{ of HCL} \times 14 \times 100}{\text{Weight of the sample taken} \times 1000}$$

S - Sample titre value
B - Blank titre value

Protein content (g) = Per cent nitrogen x 6.25

In Vitro Protein Digestibility

In vitro protein digestibility (IVPD) method was developed to stimulate the conditions in the digestive tract of human gastro intestinal tract. This method has the potential to give useful measures of *in vivo* amino acid and protein digestibility for humans. This was estimated according to the procedure of Singh and Jambunathan (1981).

An amount of sample containing 6.75±0.1mg nitrogen was placed in a 50ml conical flask and 5ml of pepsin solution was added. The flask was incubated in a water bath shaker for 16hr at 37^{0}C. Then 2ml of pancreatin solution was added and the contents were further incubated for 24hrs at 37^{0}C. Then 2 to 3 drops of toluene was added during incubation and samples were stirred slowly on a mechanical shaker. After 24hrs, the reaction was stopped by adding 20ml of 10% of TCA and the suspension was centrifuged. The residue was washed twice with 5% TCA. An aliquot of 5ml was taken and evaporated to dryness at low temperature (80-90^{0}C) and nitrogen content was determined by the Micro Kjeldal procedure. Then digestion of each sample was calculated in the following way.

$$\text{IVPD} = \frac{(\text{N in sample supernatants} - \text{N in Blank})}{\text{N in starting material}} \times 100$$

RESULTS AND DISCUSSION

The *in vitro* protein digestibility (IVPD) of finger millet, foxtail millet, little millet, pearl millet, proso millet and sorghum in raw as well as prepared items namely millet rice, millet *kichidi* and millet *roti* is given in table no.1 along with the analysis of variance between the products and between the millets and the same are depicted in fig.1.

The IVPD of raw millets ranged between 45.32% from finger millet to 59.92% from little millet. The IVPD of raw millets in the decreasing order was 59.92% from little millet, 58.42% from pearl millet, 57.77% from proso millet, 56.82% from foxtail millet, 53.13% from sorghum and 45.32% from finger millet, with an overall mean of 55.23±5.36%. The mean IVPD of raw millets was significantly lower than the mean protein digestibility of millet kichidi ($P<0.05$), but no significant difference was found between IVPD of raw millets and IVPD of millet rice and millet *roti*.

Within the millets, finger millet had significantly lower IVPD than that of other five millets studied, which is in agreement with the studies of Elshazali *et al.* (2011) where the IVPD of the whole raw flour was 46.43 and 51.23 percent, while that of the dehulled raw flour 50.54 and 55.28 percent for two varieties of finger millet. However, the IVPD can be higher depending upon the varieties as shown by Hag *et al.* (2002), where IVPD of two different pearl millet cultivars were reported as 72.7 and 70.4 percent. With respect to treatment effect higher IVPD was observed in the dehulled grains (54.86%) compared to whole grain. This was due to the established fact that, as the proportion of pericarp and germ material become less the IVPD improves (Duodu *et al.,* 2002; Chibber *et al.,* 1980). Dehulling decreased the anti-nutrients that interfere with the IVPD. Improvement in IVPD was likely due to reduction in antinutrients during traditional treatments (Babiker and Eltinay, 1993). High molecular weight polyphenols were known to precipitate proteins, reduce protein digestibility and produce off-coloured products (Hulse *et al.,* 1980).

The *in vitro* protein digestibility of millet rice, which was prepared by boiling millet in edible form as dehulled whole grain or coarse semolina ranged between 56.25% from finger millet meal to 60.83% from proso millet meal. The IVPD of millet *rice* in the decreasing order was 60.83% from proso millet meal, 58.07% from pearl millet meal, 57.77% from little millet meal, 57.23% from sorghum meal, 57.14% from foxtail millet meal and 56.25% from finger millet meal. The overall mean IVPD of millet meal was 57.88 ± 1.57%, which was significantly lower than millet *kichidi* (P<0.01), with no significant difference from IVPD of millet *roti* and raw millet. Crushing of finger millet before cooking into millet rice helped in improving IVPD from 45.32% in raw to 56.25% (11% high), while the same procedure did not show any improvement in IVPD in pearl millet, but in crushed sorghum IVPD improved slightly after cooking from 53.13% in raw to 57.23% (4% increase). In the dehulled foxtail millet, little millet and proso millet the *in vitro* protein digestibility did not show much difference after boiling to millet meal. Earlier studies have also shown that boiling did not improve IVPD in millets.

Table 1: *In vitro* protein digestibility of millet preparations

***In vitro* protein digestibility (%)**					
Millet	**Raw millet**	**Millet rice**	**Millet *Kichidi***	**Millet *Roti***	**Millet Mean ± SD**
Finger millet	45.32	56.25	64.95	46.88	53.35 ± 9.12^{a}
Foxtail millet	56.82	57.14	68.45	63.7	61.53 ± 5.60^{bc}
Little millet	59.92	57.77	66.05	64.97	62.18 ± 4.00^{c}
Pearl millet	58.42	58.07	67.34	59.87	60.93 ± 4.34^{bc}
Proso millet	57.77	60.83	73.03	65.3	64.23 ± 6.63^{c}
Sorghum	53.13	57.23	61.73	54.13	56.56 ± 3.87^{ab}
Product Mean ± SD	55.23 ± 5.36^{a}	57.88 ± 1.57^{a}	66.93 ± 3.78^{b}	59.14 ± 7.33^{a}	

Note: From ANOVA, C.D of Millets @ 1% level = 5.07**; C.D of Products @ 1% level=4.13**

The IVPD of millet *kichidi* ranged between 61.73% from sorghum millet and 73.03% from proso millet. The IVPD of millet kichidi in the decreasing order was 73.03% from proso millet, 68.45% from foxtail millet, 67.34% from pearl millet, 66.05% from little millet, 64.95% from finger millet and 61.73% from sorghum, with an overall mean protein digestibility of 66.93±3.78%. The mean IVPD of millet *kichidi* was significantly higher than IVPD of raw millet, millet *rice* and millet *roti* (P<0.01). Presence of legume in millet *kichidi* increased the total protein and supplemented the missing amino acids which has improved the IVPD of millet *kichidi. Kichidi* made from dehulled foxtail millet, little millet and proso millet had better IVPD compared to sorghum, finger millet and pearl millet with intact pericarp and anti nutritional factors.

Proso millet *roti* showed highest IVPD (65.30%), followed by little millet roti (64.97%), foxtail millet roti (63.70%), pearl millet *roti* (59.87%), sorghum *roti* (54.13%) and finger millet *roti* (46.88%) in the decreasing order of protein digestibility. The overall mean IVPD of *rotis* made of 6 millets was 59.14±7.33%, which was significantly lower than IVPD of millet kichidi (P<0.01). No significant difference was observed between IVPD of millet *roti* and raw and boiled millet (P<0.01). The *in vitro* protein digestibility of proso millet and little millet *rotis* was high by 1 and 4% compared to foxtail and little millet (all three dehulled) and by 5%, 11% and 18% compared to pearl millet, sorghum and finger millet *rotis* respectively, which were made of whole grain flours. Hima Bindu and Sumathi (2003) reported high IVPD in common Indian traditional products namely *muruku, chegodi, dosa, chapathi, laddu* and *payasam* prepared from foxtail millet.

The *in vitro* protein digestibility displayed in radar chart (Fig.1) shows the performance of different millets in different preparations by displaying which product and which millet was scoring high IVPD or low IVPD.

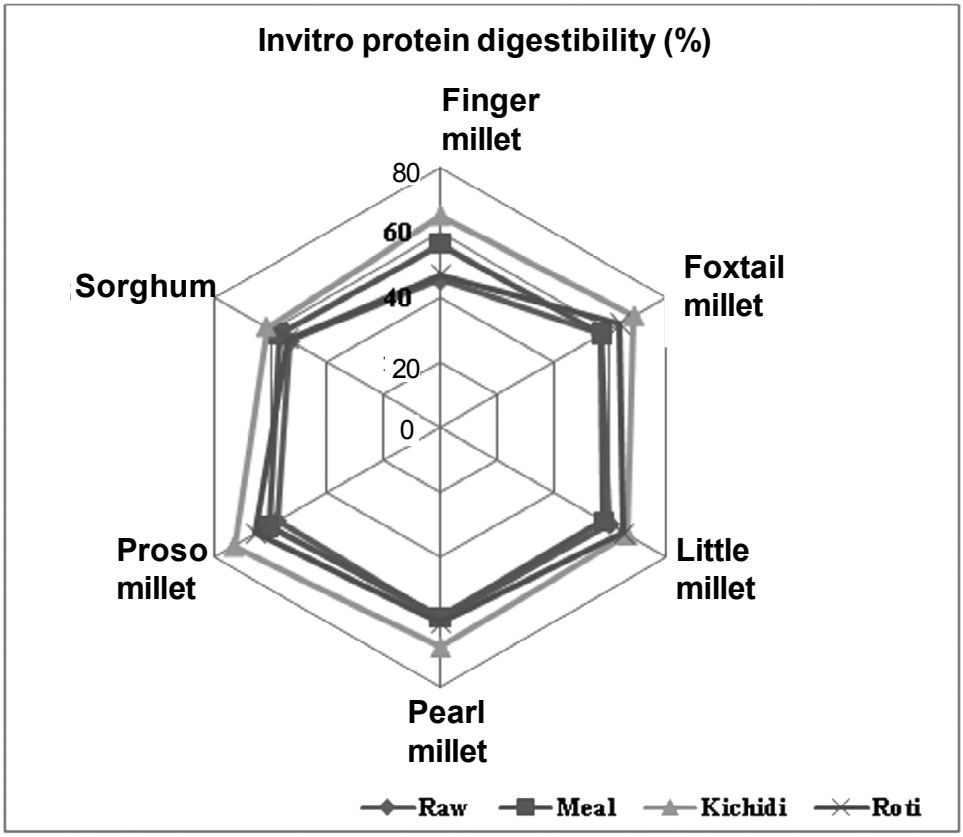

Fig.1. Spread of IVPD of millet preparations

Among the millets, the mean IVPD of finger millet products was the least with no significant difference from sorghum, but significantly low compared to mean product's IVPD of each of foxtail millet, little millet, pearl millet and proso millet ($P<0.01$). Devi *et al*. (1997) reported 76% IVPD for raw *rice* and also reported that parboiling decreased IVPD to 66% and re-cooking of the parboiled grains led to a further decrease of IVPD to 55%.

CONCLUSION

Millets made into cereal based traditional food like rice*, kichidi* and *roti* are liked by most people. Millets have gained a momentum nationwide, as healthy foods since the last one to two decades. Millets are comparable or superior to some commonly consumed cereals like wheat and rice. The *in vitro* protein digestibility of millet *kichidi*, millet *rice* and millet *roti* proved millets to be the promising substitutes for refined cereal processed foods.

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Research Article
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EFFECT OF COUNSELLING ON CONFLICT RESOLUTION SKILLS OF YOUNG ADULTS

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ABSTRACT

Conflict refers to some form of friction or discord arising between two individuals or within a group of members when the beliefs or actions between them are unacceptable. Men and women deal with conflicts differently. Inability of dealing conflicts often lead to distress affecting their wellbeing. The current study aimed, to find out the gender differences in conflict resolutions skills of young adults before and after counselling. To conduct the study a sample of 120 young adults belonging to the age group of 20-40 years were selected in the year 2016-17 to 2017-18. A self-measured conflict resolution questionnaire was used to analyse young adults conflict resolution skills. Highly significant differences were found between the genders with respect to emotional, financial, academic and career domains before and after counselling.Results revealed that men had better conflict resolution skills compared to women. However, there were improved mean scores noted in both genders after counselling resulting in enhanced conflict resolution skills.

Conflict arises from differences between two people or a group. It occurs as a result of disagreed value, beliefs, motivation, ideas or desires. These differences often lead to emotional turmoil and discrepancies in well-being of young adults. Conflicts demand more energy and unresolved conflicts will call for tremendous attention. In the modern society, young adults are fed information which puts pressure on them to meet needs that are often unrealistic. This leads to feeling of low security, low self-confidence and esteem resulting in depression, anxiety and frustration. It further affects the thought process of individuals and leading them to adapt to unsocial behaviours triggering conflicts. Unhealthy ways of dealing with conflicts often puts individuals in struggle due to irreparable rifts, resentments and breakups. On the contrast, healthy ways of conflict resolution lead to calm, non-defensive and respectful reactions. Conflict resolution skills helps individuals to manage varied situation in day-to day life. It is the ability to understand the other persons point of view and empathizing based on the situation. It also helps in build strong relationships and a whole new level of trust which is fulfilling.Effective problem-solving can increase situational coping and behavioural competence, which in turn may prevent or reduce emotional distress (D'Zurilla & Nezu, 2007).

Men and women deal with conflicts differently. They often think and deal with situations differently. This might be the result of cultural and social expectations where women are more emotional focussed and men are problem focussed in dealing with conflicts.Holt and DeVore (2005) study on conflict resolution skills revealed that males in individualistic cultures reported higher levels of conflict resolution skills compared to females. However, both males and females had poor conflict resolution skills when they are in distress. In order to address the issue,it was identified that counselling would be an effective method in facilitating young adults with improved conflict resolution skills. Conflict resolution skills are trained by the counsellor to the young adults who struggle or lack with poor conflict resolution skills. Sotsky et al., (1991) found that low levels of cognitive distortions predicted greater treatment response for patients who received Cognitive Behaviour therapy. Counselling also enables individuals to manage their emotions and better manage their conflicts. Problem Solving skills aim to strengthen positive problem orientation and reduce negative problem orientation (D'Zurilla & Nezu, 2007).Therefore, the current study aimed to understand the gender differences in conflict resolution skills among young adults before and after counselling in order to see the effectiveness of counselling.

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MATERIAL AND METHODS

Young adults in the age group of 20-40 years were selected from twin cities of Hyderabad and Secunderabad, for the study. Young adults who had approached counselling centres in order to seek help from professionals for their conflicts and stress related problems were considered as sample for the study using purposive sampling technique. Equal sample of 60 men and 60 women were selected for the study. A self- measured conflict resolution skill questionnaire was used to measure conflict resolution skills among young adults before and after counselling. The Questionnaire consists of 60 statements pertaining to 6 domains that needs to be responded to on a five-point rating scale ranging from very low to very high. The sum of marks is obtained for the entire scale. Higher the scores better is the conflict resolution skills. Spearman Brown formula was used and found that the questionnaire is highly significant (r=0.87) at 0.01 level of probability indicating high internal consistency of the conflict resolution skills.

RESULTS AND DISCUSSION

Table-1. Mean differences in conflict resolution skills based on gender (pre-test)

S.No	Dimensions	Men		Women		t-value	Probability
		Mean	SD	Mean	SD		
1.	Physical Domain	19.83	7.43	17.85	6.21	1.59 NS	0.12
2.	Emotional Domain	20.55	7.06	17.22	6.25	2.74 *	0.01
3.	Family Domain	19.08	7.37	21.23	7.42	1.59 NS	0.11
4.	Financial Domain	25.38	8.99	20.65	8.30	3.00 **	0.00
5.	Academic and Career Domain	22.70	8.65	19.10	7.88	2.38*	0.02
6.	Problem solving skills Domain	18.67	6.61	16.78	6.69	0.10 NS	0.92
	Total Scores	126.21	36.58	112.83	30.55	1.84 *	0.04

Note: *Significance at (P<0.05), **Significance at (P<0.01), NS- Not Significant

Table-1 reveals the gender differences with respect to conflict resolution skills before counselling. Highly significant gender differences were found with regard to financial, emotional, academic and career domains at 0.01 and 0.05 level of significance. Significant difference was also found in total scores of conflict resolution skills of men and women at 5% level. No significant gender differences were found with regard to physical, family and problem solving skills domains. However, mean differences existed in physical and family domain between the genders.

With respect to physical domain there were no significant differences. This might be because when in stress both men and women had experienced lack of energy tiredness and were unable to concentrate on their health aspects resulted in lower scores on physical domain. However mean differences were seen between the gender. Women had low mean scores compared to men.This might be due to socio-cultural factors such as career, responsibility towards family, biological factors such as hormonal imbalances. Post-natal depression is believed to affect eight to fifteen percent of women after they give birth. The results are in congruence with study conducted by CDC, 2009; Haskell et al. (2007) reported significant differences between activity levels, indicating that women have lower levels of activity as compared to men.

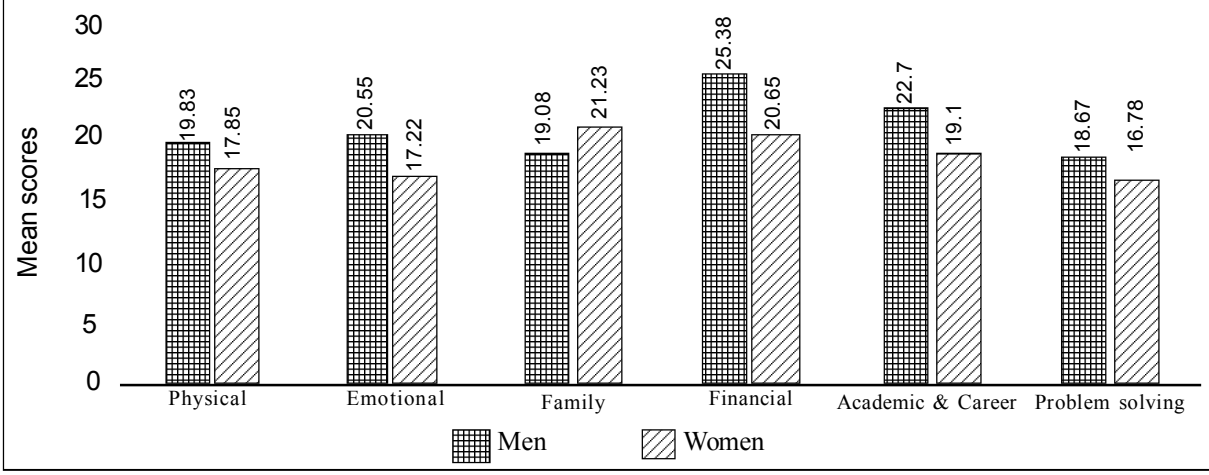
With regard to family domain women had higher mean scores compared to men. Low mean score depicts that men didn't find much support from their families and they often avoid conflicting situations at home. This also mean that men are least concerned about household tasks as they feel that it is a

woman's job to perform. On the contrary, women scored high on family domain shows that they choose family above all. This might be due to the socialization practices where women are expected to take care of the family and put their personal interests and career after family. This might also be because, women always are dependent in making decisions since childhood, right from their academic and career choices to choosing a life partner.

Low mean scores on emotional domain for women indicate that women were more emotional, anxious and worried compared to men. This might be due to the hormonal changes that occur during young adulthood period, such as reproductive events, balancing work and family life. Women are more likely to suffer depression than men. Women are more sensitive and nurturing by nature and value personal relationships compared to men. The results are in congruence with study conducted by Valentino *et al.,* (2013) revealed that the brains of females are more sensitive to a hormone produced at times of anxiety and thought to be involved in stress management. They also found that women are more sensitive to low levels of "corticotrophin-releasing factor" (CRT) but less able to cope when levels are high.

With regard to financial domain men had higher mean scores compared to women. Low scores of women might be as majority of them are homemakers dependent on their partners and lack recurring income and financial independence. This might be because men are encouraged to learn how to invest and make money grow while women are socialized to save money. This might also be differences they get paid at work and the career choices. The results are in similar to the study conducted by Natalie and Jonathan (2015) she found that women make about 79% of what men do for the same work, according to a 2014 comparison of the median earnings of full-time, year-round workers. A gap exists for highly educated workers: A 2015 Bloomberg analysis found that while men and women made similar salaries when they graduated from business school in 2007 — $105,000 for men and $98,000 for women — by 2014 those same workers had seen their salaries diverge. The men were earning $175,000, compared with $140,000 for the women.

Fig .1 . Mean differences in conflict resolution skills based on gender before counselling



From Figure-1 it was evident that with regard to academic and career domain men had higher mean scores compared to women. This might be due to the patriarchal system where women are discouraged to pursue a career of their choice. Women are socialized to take up jobs which are more secured and avoid risks while men are expected to take up a challenging career. Moreover, women are more likely to take care of their children, or look after elderly parents. The results are in line with the study conducted by Antti and Sami (2015), he found that men start their careers from higher ranks of the hierarchy than women do, although gender differences in education explain much of this gap. Men are also more likely to be promoted than women, especially during the first years in the labour market, amplifying the gender differences in hierarchical positions already apparent at labour market entry.

With regard to problem solving skills domain, no significant gender differences were found.

Negligible differences were found in the mean scores of both genders. This means that both men and women found it difficult to manage their problems when under stress. This might also be because they are unable take right decisions to solve their problems.

In total, it was clear from the above table-1 that men had higher mean scores on conflict resolution skills than women. This might be due to the tendency of women to feel more vulnerable and stressed compared to men when faced with conflicts. Women are different from men in nature. Men are more dominating and always tend to lead a dictatorship and controls reality, whereas women are socialized to be subtle and calm. This makes them take the burden and which further leads to avoid conflicts rather than to resolve. The results were in congruence with study conducted by Juliana (2013) her research found differences in the origins of disputes for men and women. Although both men and women had problems in the workplace which were associated with interpersonal relations, women reported more personality conflicts than men and seemed more sensitive to them. Women also experienced more conflicts over gender role stereotypes. Women were more likely to feel vulnerable in conflicts with men than in conflicts with other women. Women were more likely to talk about being afraid of normal conflict and of being the victim of aggression or violence. Women reported that, concerns about children, identity and status contributed to their vulnerability in conflict. Lack of support from significant others and lack of trust in the other party also reinforced feelings of vulnerability.

Table 2 . Mean differences in conflict resolution skills based on gender after counselling

S.No	Dimensions	Men		Women		t-value	Probability
		Mean	SD	Mean	SD		
1.	Physical Domain	25.65	8.51	23.13	8.06	1.663 NS	0.099
2.	Emotional Domain	30.47	9.28	25.85	9.63	2.674**	0.001
3.	Family Domain	25.77	8.15	28.10	8.14	1.569 NS	0.119
4.	Financial Domain	29.58	9.35	25.98	8.72	2.182*	0.031
5.	Academic & Career Domain	28.22	9.66	23.85	9.60	2.484*	0.014
6.	Problem solving skills Domain	28.67	9.70	27.43	9.83	0.692 NS	0.032
	Total Scores	168.36	40.72	158.86	27.99	2.014*	0.046

Note: *Significance at (P<0.05), **Significance at (P<0.05), NS- Not Significant

The above table-2 depicts the gender differences among men and women with reference to conflict resolutions skills after counselling. Significant gender differences were found with respect to emotional, financial, academic and career domains after counselling. Significant differences were also noted with reference to total scores on conflict resolution skills. No significant gender differences were found with respect to physical, family and problem solving skills domains.

With regard to physical domain (table-2) no significant gender differences were found after counselling. However, mean scores depict that men had slightly scored higher on physical domain compared to women. This might be because, both men and women equally were able to manage and concentrate on their physical health after counselling irrespective of their gender. Counselling facilitated young adults to be self- aware and improved eating and sleeping habits, this has resulted in enhanced physical domain after counselling. The results are similar to the study conducted by Galper *et al.,* (2006) they stratified their cross-sectional analysis of depressive symptoms and regular physical activity by gender and found relative increase in mental well-being associated with fitness level and reported activity, but they also found that the association did not differ for men and women.

Significant gender differences were noted with reference to emotional domain. Women had lower mean scores compared to men. This means that women were more vulnerable to their problems and this affected their emotional stability compared to men. This might be due to the interdependent nature of women and independent nature of men.It is also due to the socially acceptable display of emotions, with regard to gender are trained in the culture since early childhood period where men are not supposed to cry and dispose their emotions which might have made them to score low on emotional domain. However, both genders had considerably higher mean scores compared to pre-test. The results are further supported by Kelley & Hutson Comeaux (2002) where it was found that that the emotions of happiness, sadness and fear are believed to be more characteristic of women, whereas men are believed to be more characteristically angry.

There was no significant gender differences found with respect to family domain. However mean scores depict that men scored lower on family domain compared to women after counselling. This might be due to culturally defined roles and responsibilities of women in the work and family. Traditionally, for women the most responsibilities and demands can be found in the family domain. Cooking, cleaning, shopping, preparing meals and more tasks of this kind are womens responsibility. The expectations from the gender ideology theory point in the same direction. This theory states that women want to meet their family demands because this gives them their female identity (Milkie & Peltola, 1999). Besides this, taking care of children is mainly a women's responsibility, which has resulted in high mean scores for women than men. On the other hand, more responsibilities and demands are found in the work domain for men, as they are the provider of income for the family.

With reference to financial domain significant gender differences were found. Women had lower mean scores compared to men. Lower mean scores depict that women had less decision making roles

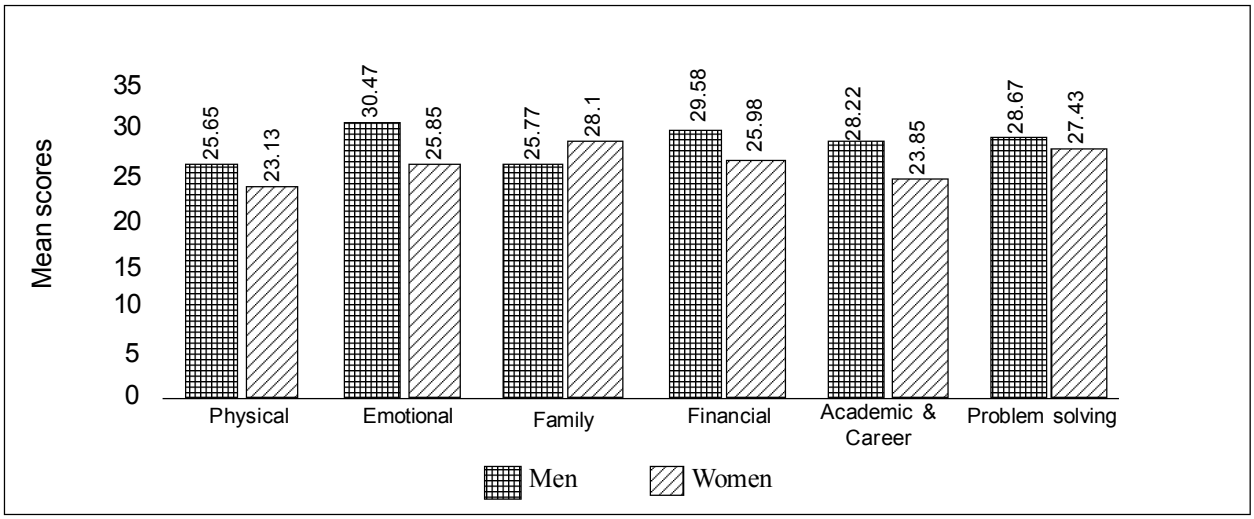


Fig. 2. Mean differences in conflict resolution skills based on gender after counselling

with regard to financial matters as that role in the family is fulfilled by men. As women had less financial independence compared to men where they handover the earnings to men and allow them to take necessary actions for the same. However, the means scores of genders had improved after counselling.

With regard to academic and career domain, significant gender differences were found. Women scored lower on academic and career domain, compared to men after counselling. This might be due to the multiple roles that women need to perform at home as a nurturer and at work as an employee lead to more pressure and experience higher levels of stress. This might also be due to the workhours that men and women spend is also responsible for high scores in men than women. Inability to strike a balance might have also led to lower scores in women. The results are in similar with the study conducted by Pekkarinen and Vartiainen 2006), who found that gender differences in the likelihood of promotion are small if initial assignment is ignored. However, when men and women sharing the same initial position are

investigated, women are much less likely to be promoted than men. Ransom and Oaxaca (2005) found gender differences in initial positions.

From the figure-2 it was evident that there were no significant gender differences with respect to problem solving skills. However, there were slight differences found in the mean scores of both genders. Men had higher mean scores compared to women. This might be because men and women react to the problems differently. Women try to solve problems by discussing with close friends or family members these discussions may sometimes get affected when the other person is disinterested and unhelpful. Whereas men approach problem with less communication and try to demonstrate their knowledge and ability in solving.

In total scores, there were significant gender differences found in post test scores after counselling. Mean scores of men were higher compared to women. This means that men had higher conflict resolutions skills compared to women. Low scores of women might be due to the intensity of chronic stress that they perceived. The competitive pressure they succumb to prove their efficiency would also had led to lower scores compared to men. Moreover, as said earlier women focus more on emotion based strategies while men focus on solution based strategies.

CONCLUSION

The results of the study revealed that significant gender differences were noted in conflict resolution skills. Women had low conflict resolution skills compared to men before and after counselling. However, improved mean scores were observed between the genders after counselling. The techniques used in the counselling session had made them build a strong routine facilitating them to be more conscious about their self. It facilitated young adults to understand their strengths and weaknesses. Such awareness had given young adults to focus on best possible solutions for their problems and diminished their anxiety and depression irrespective of their gender. Counselling was effective in preparing people to resolve their conflicts more skillfully, effectively and peacefully.

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Research Note
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EFFECT OF P^H OF GROWTH MEDIUM ON ANTIBIOSIS AND GROWTH PROMOTION ACTIVITIES OF FLUORESCENT PSEUDOMONADS

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Fluorescent pseudomonads play an important role in the prevention of plant diseases and promote the plant growth by suppressing pathogenic microorganisms, synthesizing growth stimulating plant hormones and increasing disease resistance (Stephane *et al.,* 2005). They play a dual role both in plant growth promotion and disease control (Basha *et al.,* 2012). Disease control involves direct and indirect mechanisms. Direct antagonism by production of diffusible or volatile antibiotic compounds is considered to be a primary mechanism of biocontrol. Indirectly, the pseudomonad bacteria stimulate defence responses in the plant hosts. They promote plant growth directly by production of growth promoting substances like Indole acetic acid (IAA), gibberillic acid and by making the nutrients in soil available to plants through a mechanism like phosphate solubilization (Bhattacharyya and Ghosh, 2007). Indirect plant growth promotion occurs through elimination of pathogens by production of secondary metabolites such as phenazines, phloroglucinols and cyanides (Gutterson *et al.,* 1986; Yoshihisa *et al.*, 1989;) and other major mechanisms like production of siderophores, which can form complexes with iron and make it unavailable to plant pathogens (Manwar *et al.*, 2000).Their efficacy in plant growth promotion and in their biocontrol potential found to vary based on physico-chemical properties of soils like soil type, salinity, texture, pH, temperature and moisture and other (Sangeetha *et al.*, 2011 and Loper *et al.*, 1985). The promising *P. fluorescens* isolates which were found to suppress *Sclerotium rolfsii* mycelial growth *in vitro* by dual culture assay were evaluated for understanding the influence of pH of growth media on their plant growth promotion and antagonistic potential against *S. rolfsii* and the results are presented in this paper.

This experiment was conducted in Agriculture college, Bapatla. Twenty eight *Pseudomonas fluorescens* isolates were isolated from rhizosphere of eleven cropping systems in black clay and sandy soils in Guntur district. Four isolates designated as BN, SPA, CC and CHPE showed the highest inhibition of radial growth of *S. rolfsii* in dual culture experiments. These four isolates were tested for their antagonistic and growth promoting activities like HCN, P-solubilization, IAA production under different pH of growth media viz., 5.0, 6.0, 7.0, 8.0 using paired plate technique both in presence and in absence of pathogen. Siderophore production was tested under 6.6, 6.8, 7.0 and 7.2. The pH levels of growth media were adjusted by adding NaOH and/ or HCl. Succinate agar, Pikovskay's agar and CAS agar used to test HCN, IAA, P-solubilization and siderophore production respectively. HCN production recorded based on the change in colour of filter paper from yellow to brown and OD values were also recorded at 515 nm to quantify the intensity of colour developed which corresponds to the quantity of HCN produced. Development of orange colour zone around the bacterial colony indicates positive result for siderophore production. IAA production was determined. OD values were recorded at 535 nm in spectrophotometer (Ritika *et al*, 2012). Formation of halo zone around the bacterial colony indicates production of P Solubilization.

There was no perceptible change in the colour of yellow filter papers impregnated with alkaline picric acid either in the presence or absence of *S. rolfsii* at any pH. However, OD values for colour of filter papers bleached into distilled water reflected production of minute quantities of HCN both in the presence and in the absence of pathogen. Some isolates produced more HCN in the presence of pathogen while some other isolates produced more in the absence of pathogen than other isolates. This indicates that HCN production is a constitutive character and hence is independent of the interaction of isolates with the pathogen. OD values indicating HCN production, though minute, increased with an increase in pH of

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the medium from 5.0 to 8.0 both in the presence and absence of *S. rolfsii*. All the *P. fluorescens*isolates produced the highest amounts of HCN at pH 8.0. Isolate BN followed by CC recorded significantly higher mean OD values than other isolates over all pH levels both in the presence and absence of pathogen. At pH 8.0 in the presence of the pathogen BN recorded significantly highest OD value while in the absence of pathogen CHPE recorded the highest OD value at the same pH (Tables:1and 2). Cyanogenesis by *P. fluorescens* and *P. aeruginosa*at pH between 6.6 and 8.9 was observed by Askeland and Morrison (1983).

Table 1. OD values reflecting the production of HCN by *P. fluorescens* isolates in the absence of *S. rolfsii* at different p^H levels

	p^H levels				
Isolates	**5.0**	**6.0**	**7.0**	**8.0**	**Mean**
BN	0.0430	0.0700	0.0767	0.0877	0.0693
SPA	0.0413	0.0570	0.0593	0.0730	0.0577
CC	0.0490	0.0517	0.0650	0.0710	0.0592
CHPE	0.0347	0.0463	0.0653	0.0897	0.0590
Check	0.0330	0.0303	0.0310	0.0303	0.0312
Mean	0.0402	0.0511	0.0595	0.0703	
CD (Pd"0.05) for p^H			0.0007		
CD (Pd"0.05) for isolates			0.0008		
CD (Pd"0.05) for isolates x p^H			0.0033		
CV (%)			6.2824		

Table 2. OD values reflecting the production of HCN by *P. fluorescens* isolates in the presence of *S. rolfsii* at different p^H levels

	p^H levels				
Isolates	**5.0**	**6.0**	**7.0**	**8.0**	**Mean**
BN	0.0437	0.0697	0.0773	0.0900	0.0702
SPA	0.0433	0.0587	0.0603	0.0697	0.0580
CC	0.0503	0.0537	0.0647	0.0723	0.0603
CHPE	0.0363	0.0447	0.0670	0.0863	0.0586
Check	0.0313	0.0313	0.0330	0.0303	0.0315
Mean	0.0410	0.0516	0.0605	0.0697	
CD (Pd"0.05) for p^H			0.0006		
CD (Pd"0.05) for isolates			0.0008		
CD (Pd"0.05) for isolates x p^H			0.0031		
CV (%)			5.8693		

Observations on the effect of volatiles, particularly HCN on the growth of *S. rolfsii* indicated that *S. rolfsii* radial growth in the presence of *P. fluorescens* isolates was significantly lesser than in the control plate after three days of incubation at all p^H levels. It could be elucidated that the marginal

quantities of HCN and probably other volatiles produced by *P. fluorescens* isolates might have delayed the initiation of growth of the pathogen but might have been insufficient to have a sustained inhibitory effect on the pathogen. Maximum reduction in radial growth of *S. rolfsii* was obtained with isolate CHPE which was significantly the highest. Significant highest reduction was recorded at pH 8.0 for all the isolates.

Siderophore producing ability of the *P. fluorescens* isolates at pH 6.6, 6.8, 7.0 and 7.2 was tested on chrome azurolsulphonate agar medium. Formation of an orange zone around the colony of the *P. fluorescens* isolates is considered positive for siderophore production. Orange zone was observed only at pH 6.8 in isolates BN and CC indicating that siderophore production is both pH and isolate specific. Diameter of the orange zone was significantly larger around BN colony (1.40 cm) than that of the CC isolate (1.27 cm) in the presence of the pathogen (Table:3). The two *P. fluorescens* isolates were also found to produce siderophores but at a lesser quantity in the absence of pathogen as the corresponding diameters for BN and CC isolates were 0.67 cm and 0.37 cm, respectively (Table: 4). Larger diameter corresponding to higher quantity of siderophores in the ambience of the pathogen in the paired plate could hypothetically be due to perception of certain volatile cues from the pathogen which needs to be verified. Bholay *et al.* (2012) and Sayyed *et al.* (2004) observed siderophore production at pH ranging between 4.5 and 10.0 with a peak at 7.0 but on culture media other than CAS agar with which siderophore production becomes pH specific (Shiva Kumar, 2007). Highest reduction in pathogen growth was observed with the isolate CHPE for all pH levels and pH 7.2 for all isolates which revealed that the *P. fluorescens* isolates had some inhibitory effect probably because of production of volatiles perhaps including HCN on CAS agar medium also.

Table 3. Diameter (cm) of orange coloured zone indicating siderophore production by *P. fluorescens* isolates in the presence of *S. rolfsii* at different pH levels

	p^H levels				
Isolates	**6.6**	**6.8**	**7.0**	**7.2**	**Mean**
BN	0.00 (1.0000)	1.40 (1.5483)	0.00 (1.0000)	0.00 (1.0000)	0.35 (1.1371)
SPA	0.00 (1.0000)	0.00 (1.0000)	0.00 (1.0000)	0.00 (1.0000)	0.00 (1.0000)
CC	0.00 (1.0000)	1.27 (1.5045)	0.00 (1.0000)	0.00 (1.0000)	0.32 (1.1261)
CHPE	0.00 (1.0000)	0.00 (1.0000)	0.00 (1.0000)	0.00 (1.0000)	0.00 (1.0000)
Check	0.00 (1.0000)	0.00 (1.0000)	0.00 (1.0000)	0.00 (1.0000)	0.00 (1.0000)
Mean	0.00 (1.0000)	0.53 (1.2106)	0.00 (1.0000)	0.00 (1.0000)	
CD (Pd"0.05) for p^H			0.0041		
CD (Pd"0.05) for isolates			0.0051		
CD (Pd"0.05) for isolates x p^H			0.0206		
CV (%)			2.0508		

*** Figures in parentheses are square root transformed values**

Production of IAA at different pH levels of 5.0, 6.0, 7.0 and 8.0 was tested both in presence and absence of the pathogen. Pink colour development in cell free culture filtrates of *P. fluorescens* isolates on addition of ortho-phosphoric acid indicates production of IAA.However, this reaction appears to be favoured by neutral to alkaline pH at 7.0 and 8.0. The colour developed was darker at pH 8.0 compared to that at 7.0 for all the test isolates. Yellowish tinge was observed in culture filtrates of the isolates at pH 6.0 and no colour change was recorded at 5.0. OD values for all the isolates in the ambience of the pathogen and in control significantly increased as the pH was increased from 5.0 to 8.0 with the maximum

Table 4. Diameter (cm) of orange coloured zone indicating siderophore production by *P. fluorescens* isolates in the absence of *S. rolfsii* at different pH levels

	p^H levels				
Isolates	**6.6**	**6.8**	**7.0**	**7.2**	**Mean**
BN	0.00 (1.0000)	0.67 (1.2901)	0.00 (1.0000)	0.00 (1.0000)	0.17(1.0725)
SPA	0.00 (1.0000)	0.00 (1.0000)	0.00 (1.0000)	0.00 (1.0000)	0.00 (1.0000)
CC	0.00 (1.0000)	0.37(1.1684)	0.00 (1.0000)	0.00 (1.0000)	0.09 (1.0421)
CHPE	0.00 (1.0000)	0.00 (1.0000)	0.00 (1.0000)	0.00 (1.0000)	0.00 (1.0000)
Check	0.00 (1.0000)	0.00 (1.0000)	0.00 (1.0000)	0.00 (1.0000)	0.00 (1.0000)
Mean	0.00 (1.0000)	0.21 (1.0917)	0.00 (1.0000)	0.00 (1.0000)	
CD (Pd"0.05) for p^H			0.0033		
CD (Pd"0.05) for isolates			0.0041		
CD (Pd"0.05) for isolates x p^H			0.0164		
CV (%)			1.6764		

*** Figures in parentheses are square root transformed values**

at 8.0. Significantly highest mean OD value across pH levels was recorded with isolate BN followed by SPA which in turn recorded significantly higher OD value than CC and CHPE. The differences in OD values for all the isolates in the ambience of the pathogen were only marginally higher than in the absence of pathogen (Tables: 5 and 6). Sayyed *et al.* (2004) found that IAA production increased with increasing P^H from 5.0 to 8.0 in *Pseudomonas fluorescens* 2-79 strain. Though IAA was a growth promoting substance with no likely influence on radial growth of the pathogen, the radial growth in paired plate was reduced as the pH increased from 6.0 to 8.0 which might be due to the production of volatiles like HCN by pseudomonad isolates even on succinate broth used for qualitative assessment of IAA production by *P. fluorescens* isolates.

Effect of different p^H levels of the growth medium on P- solubilization ability of *P. fluorescens* was examined for four selected isolates both in presence and in absence of the pathogen. Formation of a halo zone around the bacterial colony on Pikovskay's medium was considered as positive for P- solubilization. Halo zone around bacterial colony was not observed in any of the isolates at any p^H both in the ambience and absence of the pathogen. Repeated subculturing of isolates since their isolation from rhizosphere could have resulted in the loss of P-solubilization ability (Kucey, 1983).

Minute quantities of HCN was produced and production was found to increase with increase in pH from 5.0 to 8.0 in all isolates both in the presence and in the absence of the pathogen. Bonnie *et al.,* (1992) observed better growth reduction of *Gaeumannomyces graminis* pv *tritici* at pH 8.0 due to production of HCN. Some isolates produced more HCN in the presence of pathogen while some other isolates produced more in the absence of pathogen than other isolates. This indicates that HCN production is a constitutive character and hence is independent of the interaction of isolates with the pathogen. Siderophores were produced by BN and CC isolates only at pH 6.8, which indicated that siderophore production was pH and isolate specific. Maria *et al*., (2002) observed the reduction in siderophore production as pH decreased from 7.0 to 5.5. Production of IAA increased with increasing pH. This reaction appears to be favoured by neutral to alkaline pH at 7.0 and 8.0. P solubilisation ability not observed for any isolate at any pH level.

Table 5. OD values reflecting the production of IAA by *P. fluorescens* isolates in the presence of *S. rolfsii* at different p^H levels

	p^H levels				
Isolates	**5.0**	**6.0**	**7.0**	**8.0**	**Mean**
BN	0.0223	0.0330	0.0913	0.1930	0.0849
SPA	0.0170	0.0410	0.0820	0.1860	0.0815
CC	0.0193	0.0343	0.0413	0.1323	0.0568
CHPE	0.0137	0.0273	0.0530	0.1237	0.0544
Check	0.0130	0.0100	0.0103	0.0110	0.0111
Mean	0.0171	0.0291	0.0556	0.1292	
CD (Pd"0.05) for p^H			0.0004		
CD (Pd"0.05) for isolates			0.0005		
CD (Pd"0.05) for isolates x p^H			0.0021		
CV (%)			3.7955		

Table 6. OD values reflecting the production of IAA by *P. fluorescens* isolates in the absence of *S. rolfsii* at different pH levels

	p^H levels				
Isolates	**5.0**	**6.0**	**7.0**	**8.0**	**Mean**
BN	0.0200	0.0350	0.0873	0.1923	0.0837
SPA	0.0177	0.0373	0.0813	0.1837	0.0800
CC	0.0167	0.0310	0.0357	0.1317	0.0538
CHPE	0.0173	0.0303	0.0463	0.1300	0.0560
Check	0.0113	0.0103	0.0133	0.0110	0.0115
Mean	0.0166	0.0288	0.0528	0.1297	
CD (Pd"0.05) for p^H			0.0004		
CD (Pd"0.05) for isolates			0.0005		
CD (Pd"0.05) for isolates x p^H			0.0021		
CV (%)			3.8801		

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Research Note
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CORRELATION STUDIES FOR GRAIN YIELD AND ITS COMPONENTS IN HYBRIDS OF QUALITY PROTEIN MAIZE (*Zea mays* L.)

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Maize is the third most important cereal crop after wheat and rice and is considered one of the most versatile emerging crop having wider adaptability under varied agro-climatic conditions. During 2015-16, globally maize was cultivated on an area of 178 million ha in about 160 countries with a total production of 1037 million tons (Foreign Agricultural Service, USDA, 2016). In India, the crop was grown on 9.2 million ha with a total production of 26.15 million metric tons and an average yield of 3340 kg/ha. In Telangana state, it covers an area of 0.69 million hectares with a production of 2.31 million tonnes and productivity of 3340 kg ha-1 (India Stat, 2015).

Maize is deficient in some essential amino acids such as lysine and tryptophan, provitamin A and micronutrients such as iron, zinc and calcium whose bioavailability is significantly lowered due to chelators such as phytate which is indigestible by monogastric animals, including humans (Sun *et al.,* 2011). To overcome these problems, decades of efforts by researchers at International Maize and Wheat Improvement Centre (CIMMYT) led to the development of lines with enhanced nutritional values which were designated as "Quality Protein Maize" or QPM lines (Vasal *et al.,* 1980).

Grain yield is a complex character governed by several contributing traits. Hence, character association was studied in the present investigation, to assess the relationship among yield and its components for enhancing the usefulness of selection in quality protein maize hybrids.

Eight inbred lines were crossed with three inbred testers in a line x tester mating design in summer, 2015 to generate 24 crosses. All the 24 crosses and two standard checks Vivek QPM-9 and HQPM-1and parents (lines and testers) were evaluated during *kharif*, 2016 at Winter Nursery Center (ARI), Rajendranagar, Hyderabad,Telangana State. Each genotype was grown in three rows of four meters length with 60 x 20 cm spacing in a randomized block design with three replications. The trial was conducted in sandy loam soil. All the recommended agronomic practices were followed to raise a normal crop. Data were recorded on five randomly selected plants in each treatment for ten characters viz., plant height, ear height, ear length, ear diameter, number of kernel rows per ear, number of kernels per row, 100-seed weight, shelling percentage, protein content and grain yield per plant, while days to 50 per cent tasseling, days to 50 per cent silking and days to maturity were recorded on whole plot basis. The data collected were subjected to analysis of variance as suggested by Panse and Sukhatme (1985). Correlation coefficient was calculated using the method given by Snedecor and Cochran (1989).

Character association studies will help to assess the relationship among the yield and its components for enhancing the usefulness of the selection. Genotypic correlations reveal the existence of real associations where as the phenotypic correlations without significant genotypic associations are of no value. This indicates the importance of genotypic correlation compared to the phenotypic correlation. In the present investigation, phenotypic and genotypic correlations were worked out on yield and yield contributing characters in 24 crosses and 11 parents. In general, genotypic correlations are of slightly higher magnitude than the corresponding phenotypic values and hence only the genotypic correlations are discussed here under table 1.

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Table 1: Estimates of phenotypic and genotypic correlation coefficients among yield attributing characters

Trait		DTT	DTS	DM	PH(cm)	EH(cm)	EL(cm)	EG(cm)	KRPE	KPR	SW	SP	PC %	GY
DTT	P	1.00	0.77**	-0.12	-0.31**	-0.15	-0.19	-0.19	-0.30**	-0.24*	-0.16	-0.077	-0.13	-0.27*
	G	1.00	-0.45**	-0.28*	-0.48**	-0.18	-0.35**	-0.59**	-0.62**	-0.56**	-0.08	-0.12	-0.22	-0.48**
DTS	P		1.00	-0.10	-0.22	-0.04	-0.20	-0.24*	-0.28*	-0.24*	-0.06	-0.11	-0.07	-0.24*
	G		1.00	-0.17	-0.34**	-0.09	-0.28*	-0.48**	-0.58**	-0.44**	-0.13	-0.20	-0.12	-0.38**
DM	P			1.00	0.23*	-0.08	0.27*	0.31**	0.28*	0.32**	0.14	-0.14	0.38**	0.15
	G			1.00	0.43**	0.02	0.40**	0.61**	0.38**	0.48**	0.20	-0.14	0.56**	0.23*
PH	P				1.00	0.63**	0.48**	0.38**	0.47**	0.52**	0.25*	0.23*	0.52**	0.45**
	G				1.00	0.77**	0.63**	0.54**	0.59**	0.63**	0.28*	0.24*	0.56**	0.50**
EH	P					1.00	0.13	-0.002	0.08	0.10	-0.006	0.01	0.21	0.26*
	G					1.00	0.23*	0.10	0.19	0.19	-0.08	0.01	0.25*	0.31**
EL	P						1.000	0.57**	0.55**	0.74**	0.24*	0.18	0.52**	0.58**
	G						1.00	0.83**	0.81**	0.93**	0.41**	0.22	0.62**	0.71**
EG	P							1.00	0.60**	0.59**	0.49**	0.18	0.51**	0.53**
	G							1.00	0.87**	0.94**	0.71**	0.24*	0.68**	0.73**
KRPE	P								1.00	0.69**	0.33**	0. 16	0.53**	0.57**
	G								1.00	0.91**	0.59**	0.22	0.66**	0.72**
KPR	P									1.00	0.24*	0.09	0.63**	0.66**
	G									1.00	0.50**	0.13	0.71**	0.78**
SW	P										1.00	0.36**	0.28*	0.52**
	G										1.00	0.46 **	0.37**	0.71**
SP	P											1.00	-0.11	0.17
	G											1.00	-0.11	0.18
PC %	P												1.00	0.50**
	G												1.00	0.52**
GY	P													1.00
	G													1.00

P represents Phenotypic correlation coefficient; G represents Genotypic correlation coefficient. *Significant at 5 per cent level; ** Significant at 1 per cent level. DTT- Days to 50% of tasseling ; DTS - Days to 50% silking; DM - Days to maturity ; PH - Plant height ; EH- Ear height ; EL- Ear length; EG- Ear girth; KRPE –Number of Kernel rows per ear ; KPR- Number of Kernels per row ; SW-100 Seed weight ; SP- Shelling percentage; PC %- Protein content in %, GY - Grain yield per plant.

Grain yield per plant was found to be significant and positively associated with days to maturity, ear length, number of kernels per row, plant height, ear height, ear girth, number of kernel rows per ear, 100-seed weight and negatively associated with days to 50 per cent tasseling and days to 50 per cent silking. These results are in confirmation with the findings of Kanagarasu *et al*. (2013), Udaya Bhanu Kote *et al*. (2014), and Bullo Neda Tulu (2014).

Number of kernels per row exhibited highest positive correlation with grain yield per plant followed by ear girth, number kernel rows per ear, ear length, 100 seed weight, protein content, ear height, plant height and days to maturity and negative significant association with days to tasselling and days to silking.

Number of kernels per row recorded significant positive correlation with days to maturity, plant height, ear length, ear girth, number of kernel rows per ear, 100-seed weight and grain yield per plant. Similar results were reported earlier in maize for the association of grain yield with number of kernels per row by Batool Zarei *et al*. (2012) and Amit Kumar *et al*. (2014).

Ear girth had significant positive correlation with days to maturity, plant height, ear length, number of kernel rows per ear, number of kernels per row, 100-seed weight and grain yield per plant. Similar results were reported earlier in maize for the association of grain yield with ear girth by Kumar *et al.* (2006), Pavan *et al.* (2011), Raghu *et al.* (2011), Ram Reddy *et al*. (2012) and Ravi *et al.* (2012).

Days to 50 per cent tasselling and days to 50 per cent silking showed significant negative association with grain yield per plant and plant height, ear length, ear girth, number of kernel rows per ear and number of kernels per row. Similar results were reported earlier in maize for negative association of grain yield with days to 50 per cent tasseling by Malik *et al*. (2005). Days to maturity exhibited significant positive association with plant height, ear length, ear girth, number of kernel rows per ear, number of kernels per row, and grain yield per plant. This trait revealed non-significant negative association with shelling percentage. Udaya Bhanu Kote *et al.* (2014) and Vijay Kumar *et al*. (2015) reported similar results.

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UTILIZATION OF EST-SSR MARKER FOR THE GENERATION OF ADDITIONAL DESCRIPTOR FOR THE IDENTIFICATION OF ELITE MATERIAL IN CASTOR (*Ricinus communis* L.)

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Oilseeds occupy a pride place in Indian economy next to food grains. Castor (*Ricinus communis* L.) plays an important role in the country's vegetable oil economy. India accounts for 59% of global castor area and 81% of world castor production and ranks first in area and production in the world.

In the regime of PPV & FR, breeders are interested in protecting their elite breeding material. Most of the breeders believe in the morphological characters while protecting their material. But morphological traits are prone to variation. Under such situation breeders look for tools which will help them to protect the material may be at DNA, RNA and Protein or biochemical level to identify the elite breeding material. Molecular markers are now widely used to identify plant varieties (Guiard, 2007) and to monitor genetic purity (Nandakumar *et al*., 2004; Tsukazaki *et al*., 2008). It is important to utilize marker systems that are reflective of genotype, highly discriminative, reliably scorable and amenable to high-throughput analysis and cost-effective (Gale *et al*., 2005). EST-SSR marker or SSR markers are routinely utilized by the breeders for varietal identification or fingerprinting. Two genotypes PCS-106 and PCS-345 were found to possess high root type and high yield characters. In the present investigation additional information was generated by utilizing EST-SSR markers for drought tolerance for the two elite genotypes of castor *viz*., PCS-106 and PCS-345.

The plant material used in this study was selected from a previous study conducted by Sagarika (2014) in an elevated root study structure where in thirty four elite castor genotypes released from Regional Agricultural Research Station (RARS), Professor Jayashankar Telanagana State Agricultural University, Palem were screened for high water use efficiency based on physiological parameters, root architectural characters and molecular diversity analysis (Plate 1).

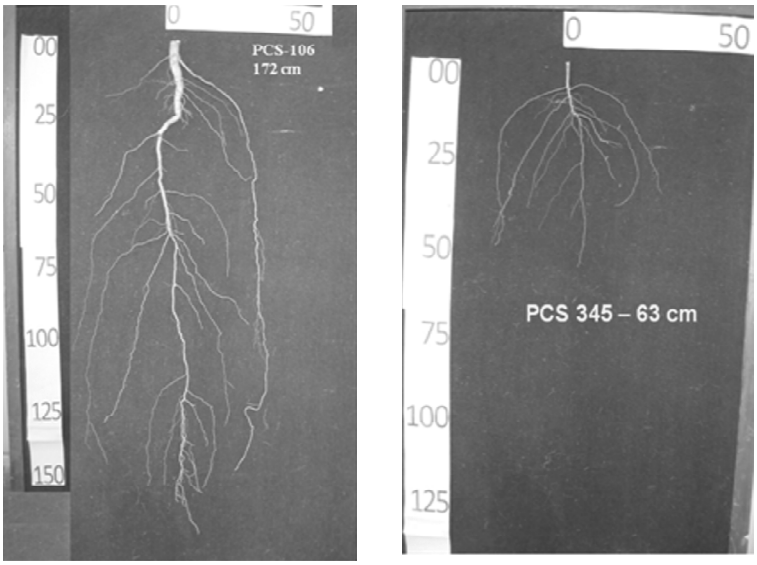

Plate 1. Root characters of PCS-106 and PCS-345

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The outcome of the study revealed that PCS-106 has recorded long roots (172 cm) with maximum root weight (45.3g) and it also showed maximum intrinsic WUE (WUE_i) of 47.86. PCS-106 also showed significant lower CID (18.26) values indicating drought tolerant nature of that genotype. It was a well established fact that the genotypes with lower CID values with high WUE are considered to be drought tolerant types (Vadez *et al.,* 2014). Accordingly a strong negative relationship between CID and WUE was established under moisture stress conditions in sunflower by Nageswar Rao and Wright (1994).

It was also noticed in the investigation that among all the genotypes studied PCS-345 recorded higher SCMR values (52.3), maximum seed weight (74 g $plant^{-1}$), maximum dry matter production (300.9 g $plant^{-1}$) and seed yield (876 g $plant^{-1}$).

From the study by Sagarika (2014) it can be suggested that the identified elite castor genotypes *viz.*, PCS-106 and PCS-345 may be recommended as donor parental material for developing specific varieties for water use efficiency or drought tolerance.

Variability was observed between these genotypes for different morphological characters *viz.*, bloom (waxy coating on aerial parts of plant), stem coloration, plant height, no. of nodes, no. of branches and nature of spike (Table 1). The diversity of the genotypes has also been recorded previously (Thatikunta *et al.*, 2014).

These genotypes were further utilized for the generation of additional information by using 38 EST-SSR primer pairs to characterize the elite genotypes (Table 1). Few of them are already reported by Thatikunta *et al.* (2016). Genomic DNA was extracted from young tender leaves from each parent following the standard cTAB method with minor modifications (Doyle and Doyle, 1987).

The DNA quantification was done by using a Nanodrop spectrophotometer (Thermo Scientific). PCR optimization for EST SSR markers was done by varying concentration of template DNA, Taq polymerase, dNTPs, primers and $MgCl_2$. The amplification reaction with EST SSR primers was carried out in a final volume of 10 µl in DNA Thermo cycler (Eppendorf Mastercycler Pro S). Each reaction mixture contained 2.0 µl 10 X reaction buffer containing 1.6 mM $MgCl_2$, 3.0 U of Taq DNA polymerase, 2 µl of 0.1 mM dNTP, 10.0 picomoles each of forward and reverse primer and approximately 50 ng/µl of template DNA. The PCR amplification conditions are as follows: initial denaturation at 94 ^{0}C for 4 min followed by 35 cycles of denaturation at 94 ^{0}C for 45 sec, primer annealing at 55 to 60^{0}C for 45 sec and elongation at 72 ^{0}C for 1 min, followed by final elongation at 72 ^{0}C for 7 min. 10 ìl of the amplified PCR product from each reaction was separated on 3.0% agarose gel (Lonza) containing ethidium bromide in 1 x TAE buffer at 120 V, finally visualized and photographed using gel documentation. In the present investigation, 38 EST SSR markers were used to check the polymorphism between the parents.

The already identified contrasting morphological traits recorded for two elite genotypes (PCS 106 and PCS-345) are presented in Table 2. It is being noted that both the genotypes are prominent and found to be contrasting for almost all listed characters. All these characters may be subjected to environmental variation. Hence, efforts were made to generate addition descriptor information for two diverse castor genotypes which possessed drought tolerance (Sagarika, 2014). The variation among these parents was characterized using 38 EST SSR markers. Out of them twelve microsatellite markers, each were showing polymorphism of 31.57% between PCS 106 and PCS-345 (Fig. 1). For most EST-SSR markers the band width ranges from 185 to 380 bp was observed. The banding pattern for each of these elite lines is unique for each of the twelve markers used in the present study and these unique bands may represent unique allele in genome (Kim *et al.*, 2012). This peculiar banding pattern for each EST-SSR can be utilized as additional information for the protection of these elite materials as EST-SSR markers are DNA based markers can be highly transferable between populations, and the data can be reproducible and exchangeable easily across laboratories (Kadirvel *et al.*, 2015). Earlier Song *et al.* (1999) identified 13 SSR loci that could be used to uniquely identify 66 elite North American soybean (*Glycine max* L.) varieties, including several varieties that had identical maturity, morphological or pigmentation traits.

The level of polymorphism studied using EST-SSRs was low which is perhaps due to higher conservation of the coding regions among genotypes with in a species (Eujayl *et al.*, 2002). However, if very closely related inbreds need to be routinely separated, much larger marker numbers are needed but in the present study both the elite lines are contrasting to each other and number of markers utilized for the identification are justifiable. Prasad *et al.* (2000) uniquely identified 48 wheat (*Triticum aestivum* L.) varieties using 12 SSR loci which supports our finding. Hence, from the above discussion we summarize that the additional information generated by utilizing EST-SSR markers can serve to identify the elite genotypes in castor.

Table 1. List of different characters of elite genotypes

S.No	Trait	PCS -106	PCS - 345
1	Stem pigmentation	Green	Red
2	Bloom	Double bloom (wax coating on stem, petiole and lower side of the leaves)	Double bloom (wax coating on stem, petiole and lower side of the leaves)
3	No. of nodes	12-14 (Medium duration)	9-10
4	Days to 50% flowering	40-44 days	35-38 days
5	Days to maturity	88-92 days during *kharif* (Primary spike)	83-85 days during *kharif*
6	Spininess of capsules	Spiny	Non-spiny
7	Duration	90-180 days	85-150 days (Early duration)
8	Resistant to wilt	Resistant	Susceptible
9	Tolerant to drought	Tolerant	Susceptible
10	Root system	Deep root (172 cm)	Shallow root (63 cm)

Table 2. List of primers used in the present study

S.No	Primer name	Sequence	Amplicon size (bp)
1.	Rc - ANGRAU -SSR1	ACGAGGCTCTGTCTCTGTGT (F) GGCAAAATTCAACACCATTC (R)	125
2.	Rc - ANGRAU - SSR 18	TTAGGGTTTGGTTCTTTGGA (F) TCAAGTGCCCATTTTAGAGC (R)	186
3.	Rc - ANGRAU - SSR 20	AGCAGCAACAAACTACGCTT (F) GTTGCATCGATAGAGCCTGT (R)	279
4.	Rc - ANGRAU - SSR - 22	AAAAGGTGGGATAGAGCCTGT (F) CCATCAAGAATACCCTCCCT (R)	400
5.	Rc - ANGRAU - SSR - 25	CTGCTTCTCTCTGCATTGTG (F) CATGGGATCTTTGGTTCTTG (R)	276
6.	Rc - ANGRAU - SSR - 33	ATTTATTGCATGTTGCAGCC (F) GCAAAGTTCACCGATGAGAA (R)	378
7.	Rc - ANGRAU - SSR - 34	AAAAGACAAAGCAGCAACCAC (F) GCAGCTCCGAGTTGTACG (R)	225

S.No	Primer name	Sequence	Amplicon size (bp)
8.	Rc - ANGRAU - SSR - 43	AACGAAGCATTTTAATCCCC (F) GTGAGCGAGATTATCGGAGA (R)	367
9.	Rc - ANGRAU - SSR - 46	TCCCATCAGTTTTTGCTGTT (F) AGAATAGGATCCATCCTGCC (R)	259
10.	Rc - ANGRAU - SSR - 6	ATACTCTCAGTGCTGCTGCC (F) AAAACGTGTAAACGGGATCA (R)	252
11.	GB RC 019	GTATGGTACGATCTCTTTGGAC (F) TACGCAGAGAAGCACTAATAGA (R)	277
12.	GB RC 021	AAGCTCAACTTAAAAGCCTAGA (F) ATTTTGGTGGTCTCTAGTTCAGTC (R)	211
13.	GB RC 080	TTGAGGAAACAGAAGATCAAAT (F) AGCACGCCAATACCTCTTGTAA (R)	213
14.	GB RC 105	TAGATTTTTATGGATAGGTGCC (F) ATGTAGACACTTGACTCACGAA (R)	206
15.	Rc DES 28	CCCAAAAGACTAACAACAACC (F) ATGCATCTGTTGGAGTTGC (R)	239
16.	Rc DES 45	CACAAACACACATATCATGTCC (F) CTCAAGTGCATCTGAAACG (R)	218
17.	Rc DES 46	ACGAGGAGGGAGACTAAATGC (F) CACTGATATACACACCACAGTGAC (R)	229
18.	Rc DES 100	CTGGCATTGCAGATCGTATGA (F) GCGCCACCACCTTGATCTT (R)	233
19.	RcDES 592	TCTCATCACTAACAAACCAGCC (F) GGTTCTGAAAGAAGTGAAATGG (R)	128
20.	RcDES 593	GTTCAAGCTAGTTCGGTGAG (F) TACACTTTCTGTCTGTCCATGC (R)	371
21.	Rc DES 594	GGCTTCTAATCTTTACTTCACC TATTTTCATCACCGACCCTAAC	235
22.	Rc DES 595	TCTCATCACTAACAAACCAGCC GGTTCTGAAAGAAGTGAAATGG	150
23.	Rc DES 596	TCCACATTGCTAACAAGCATAG AAATGCACACAGCTAAGACAAG	381
24.	Rc DES 597	CAGCCAAACATAAGATTCATGC ATCATCTGGGTGTCTCAAAATC	379
25.	Rc DES 598	GAAAGCAACAAGAAAAGGTCTG AAGCTGGTGGTTTACTTGCTAC	346
26.	Rc DES 599	CTTCATTAAGCCATCAAAGAGC AATGTAGTGTCTGTCTGTTGCG	256
27.	Rc DES 600	CCGCTCACTACAAGTGGTACTC GAGTACTGGACAGCGATGAGAC	387

S.No	Primer name	Sequence	Amplicon size (bp)
28.	Rc DES 601	TAACTTGTCTGTTTTGGGTGAC GATTGGAGTCATGGAGAGAGAG	258
29.	RcDES602	GCTCCTTTTCTTTTCTTGTGTG CTAAATGAAAACATCGGTAGCC	186
30.	RcDES603	GATTGTCTCAGCTTTTGGCTAC GCATTGCTAAGTGCAAAGTCC	314
31.	RcDES604	TACACTAATTCCTTCCAATCCC AATCATCACCACCATCAAACTC	285
32.	RcDES605	GGAGGAGAAGAAGAAAGAAACC ATGTATCAACTGGGAGACAACC	303
33.	RcDES606	CATAATTCCAGCGAACACAAAC CTTCTTAATCGACTCCAAATCG	291
34.	RcDES607	CATCTACCTAGAGCTCGAATGC GTTTAGGAGGAGATTGAGGAGG	385
35.	RcDES608	GAGCCATTTCCTCTGTATTTTC AAATCCATCTGGACAAACTGAC	365
36.	RcDES609	ACTATACAAGCAAGCAAGCAAG TTGTAGGGTTTATGTTGTTCCC	190
37.	RcDES610	CTTAGGTGGACTTAGCTCATGG AGTTCCAAAACGTCAATGTCTC	264
38.	RcDES611	GAAAATGGAGTTCCTGAACAAG CGAAAGAATCCAAAGACTTGAC	315

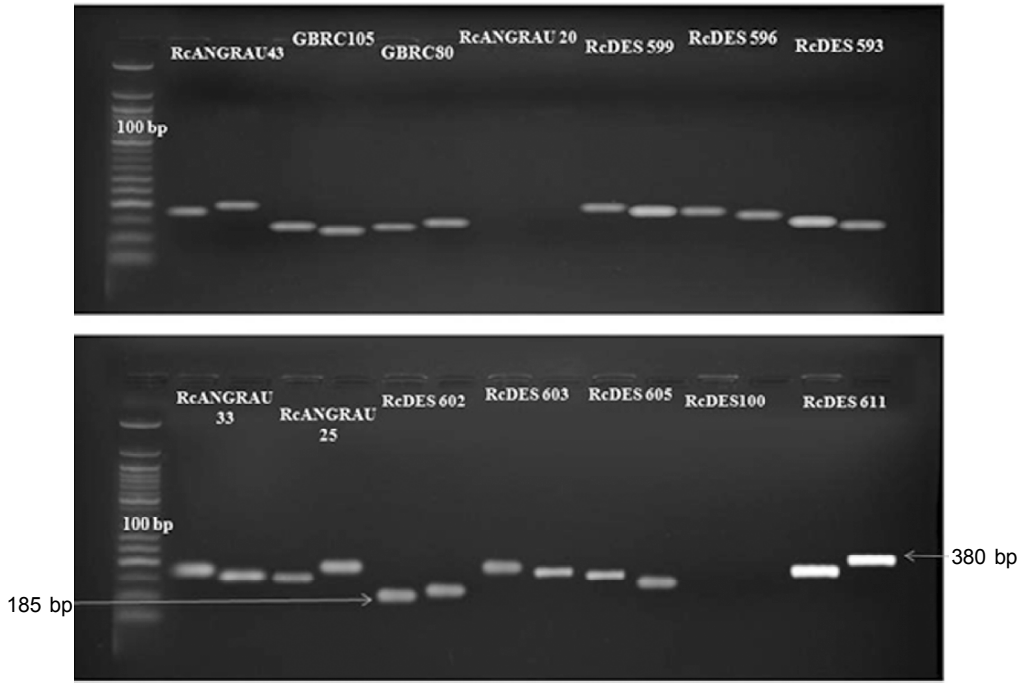

Fig 1. Segregation pattern for the two elite genotypes (PCS-106 and PCS-345) by utilizing 38 ESR-SSR marker wherein 12 markers showed polymorphism with band width of 185 to 380bp (RcANGRAU43, RcANGRAU33, RcANGRAU25, GBRC 105, GBRC 80, RcDES 593, RcDES 596, RcDES 599, RcDES 602, RcDES 603, RcDES 605 and RcDES 611).

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Research Note
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EFFECT OF N AND K FERTIGATION SCHEDULES ON YIELD ATTRIBUTES AND YIELD OF SUNFLOWER (*Helianthus annus* L.)

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Sunflower ranks third in the area and fourth in production in world. It is cultivated in an area of 18.22 million hectares with an annual production and productivity of 22.03 million tones and 1216 kg ha^{-1} respectively, in the world (Anonymous, 2015). Sunflower (*Helianthus annuus* L.) is an important oilseed crop in India. It is cultivated over an area of about 5.2 lakh hectares with a production of 3.35 lakh tonnes and productivity of 643 kg ha^{-1} (Anonymous, 2015).

The sustainability of any production system requires optimum utilization of resources like Soil, water and fertilizer. Efficient use of water and fertilizer is highly critical to sustain the agricultural production. In fertigation, nutrient use efficiency could be as high as 90 per cent compared to 40 to 60 per cent in conventional methods (Solaimalai *et al.,* 2005). Fertigation of nutrients significantly increased saving of fertilizer nutrients up to 40 per cent without affecting the yield of crops compared to the conventional method of nutrient application (Sathya *et al*., 2008).

Drip fertigation with N and K is more common when compared to P fertigation. Fertigation with P has not been widely used, mainly because emitters can get clogged by the formation of insoluble P precipitates. Further, the cost of fertilizers could be reduced to partial supply of fertilizer through fertigation or only supplying N and K through drip system. Application of fertilizers along with irrigation water through drip fertigation can improve sunflower yield and fertilizer use efficiency and meets crop demand throughout the crop growing season. Such information on drip fertigation for sunflower is scanty for Telangana state and the Government is concerning water saving and improving water and nutrient use efficiency by encouraging adoption of micro irrigation. With this background, an effort was made to do following study.

The field experiment was carried out at Water Technology Centre, College Farm, Rajendranagar, Hyderabad during *rabi*, 2017-18. The experiment was laid out in randomized block design with three replications and nine treatments at 100% RDF *viz*., T_1 control (no application of N and K fertilizers + drip irrigation, T_2 (manual application of N and K fertilizers + drip irrigation.), T_3 (application of only N (75 kg ha^{-1}) through fertigation with 4 days interval.), T_4 (application of only N (75kg ha^{-1}) through fertigation with 8 days interval), T_5 (application of only K (30 kg ha^{-1}) through fertigation with 4 days interval), T_6 (application of only K (30 kg ha^{-1}) through fertigation with 8 days interval), T_7 (application of N and K (75 kg N and 30 kg K_2O ha^{-1}) through fertigation with 4 days interval), T_8 (application of N and K (75 kg N and 30 kg K_2O ha^{-1}) through fertigation with 8 days interval) and T_9 (manual application of N and K fertilizers + furrow irrigation).

The water source for irrigation was from an open well. The irrigation water was alkaline without any residual alkalinity problem. The laterals of 16 mm diameter were laid at 1.2 m apart with spacing of 0.5 m distance between two inline emitters. The emitter discharge was 4.0 lph. The irrigation to T_1 to T_8 treatments was scheduled at 0.8 Epan and T_9 at IW/CPE of 1.0. The recommended dose of fertilizer (RDF) 75, 90 and 30 kg N, P_2O_5 and K_2O ha^{-1}, respectively was applied in the form of Urea, Single Super Phosphate (SSP), and Murate of Potash (MOP) / Sulphate of Potash (SOP). A common dose of phosphorus was applied to all the treatments including control (T_1). Nitrogen and Potassium were applied through drip fertigation at different growth stages as per treatments. For manual application treatments (T_2 and T_9) N was applied in three equal splits at basal, 30 and 50 DAS through urea and potassium was applied through MOP in a single basal dose. Fertigation was given at 4 and 8 days intervals starting from 16 DAS to 88 DAS.

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The soil was sandy clay loam, alkaline in reaction, non saline, low in available nitrogen, medium in available phosphorus and potassium. Sunflower hybrid DRSH-1 was sown by paired row system on 16th November 2017 by adopting a spacing of 80/40 cm between the rows and 25 cm between the plants to maintain a desired plant population of 66,666 plants ha^{-1}. Irrigations were scheduled based on the USWB Class A pan evaporation rates (0.8 replenishment factor) for treatments under drip irrigation (466.6 mm) and the calculated irrigation water was delivered in surface irrigation (318.8 mm) treatment plot directly using a water meter and a flexible pipe.

The results pertaining to yield attributes are in Table 1. The results revealed that at harvest, the head diameter ranged from 11.8 to 18.6 cm and the highest head diameter was observed with T_7 (N and K at 4 days interval) (18.6 cm) which was on par with T_8 (N and K at 8 days interval) (18.2 cm) followed by T_2 (manual application of N and K fertilizers + drip irrigation) (16.2 cm) which was on par with (T_3 and T_4) only N at 4 days (14.7 cm) and 8 days interval (14.5 cm). The lowest value was observed with T_1 (control) (11.8 cm). N and K at 4 days interval (T_8) has recorded 14.8% increase in head diameter compared to manual application of N and K fertilizers + drip irrigation (T_2). Manual application of N and K fertilizers + drip irrigation (T_2) has recorded 15.7% increase in head diameter compared to manual application of N and K fertilizers + furrow irrigation (T_9). Supporting results for highest head diameter by N fertigation over broadcast method of fertilizer application were reported by Buriro (2005), Gandahi (2005) in sunflower. Similar result was obtained by Himaja (2017) at 100% RD of NPK through fertigation at weekly interval in sunflower.

The number of filled seeds $head^{-1}$ ranged from 487 to 1014. The highest number of seeds $head^{-1}$ was observed with T_7 (N and K at 4 days interval) (1014) which was on par with T_8 (N and K at 8 days interval) (946) followed by T_2 (manual application of N and K fertilizers + drip irrigation) (833) which was on par with T_3, T_4, T_5, T_6 and T_7 respectively followed by T_9 (manual application of N and K fertilizers + furrow irrigation) (670). The lowest value was observed with T_1 (control) (487). The application of N and K at 4 days interval (T_7) has recorded 21.7% more number of seeds $head^{-1}$ compared to manual application of N and K fertilizers + drip irrigation (T_2). Manual application of N and K fertilizers + drip irrigation (T_2) has recorded 24.3% more number of seeds $head^{-1}$ compared to manual application of N and K fertilizers + furrow irrigation (T_9). The result was in agreement with that of Himaja (2017) with 100% RD of NPK through fertigation at weekly interval in sunflower.

The Seed yield $plant^{-1}$ (g) ranged from 18.9 to 39.3 g. The highest seed yield $plant^{-1}$ was observed with T_7 (N and K at 4 days interval) (39.3 g) which was on par with T_8 (N and K at 8 days interval) (36.5 g) followed by T_2 (manual application of N and K fertilizers + drip irrigation) (32.4 g) which was on par with T_3 (only N at 4 days interval) (29.7 g). Lowest value was observed with T_1 (control) (18.9 g). N and K at 4 days interval (T_7) has recorded 21.3% higher seed yield $plant^{-1}$ compared to manual application of N and K fertilizers + drip irrigation (T_2). Manual application of N and K fertilizers + drip irrigation (T_2) has recorded 20.4% higher seed yield $plant^{-1}$ compared to manual application of N and K fertilizers + furrow irrigation (T_9).

The Test weight (g) ranged from 46.1 to 80.3 g and significantly the highest test weight was recorded in T_7 (N and K at 4 days interval) (80.3 g) which was on par with T_8 (N and K at 8 days interval) (76.6 g) followed by T_2 (manual application of N and K fertilizers + drip irrigation) (68.3 g) which was on par with T_3 and T_5 respectively. The lowest value was observed with T_1 (control) (46.1 g). The N and K at 4 days interval (T_7) has recorded 17.6% higher test weight compared to manual application of N and K fertilizers + drip irrigation (T_2). Manual application of N and K fertilizers + drip irrigation (T_2) has recorded 17.1% higher test weight compared to manual application of N and K fertilizers + furrow irrigation (T_9). The result was relavent to the findings of Himaja (2017) with 100% RD of NPK through fertigation at weekly interval in sunflower.

The results are presented in Table 2. Significantly the highest seed yield (2623 kg ha^{-1}) is obtained with the application of N and K at 4 days interval (T_7) which was on par with T_8 (N and K at 8 days interval) (2437 kg ha^{-1}). The seed yield realized with T_8 was found to be at par with T_2 (manual application of N and K fertilizers + drip irrigation) (2158 kg ha^{-1}). The lowest seed yield was observed with T_1 (control) (1262 kg ha^{-1}). The N and K at 4 days interval (T_7) has recorded 21.5% higher seed yield compared to manual application of N and K fertilizers + drip

irrigation (T_2). Manual application of N and K fertilizers + drip irrigation (T_2) has recorded 20.2% higher seed yield compared to manual application of N and K fertilizers + furrow irrigation (T_9).

From the above results it can be concluded that the highest seed yield was obtained with the application of N and K through fertigation with 4 and 8 days intervals over soil application method. Frequent application of nutrients with sufficient quantity of water resulted in the maintenance of adequate soil moisture level in the crop root zone and sufficient amount of nutrients in the soil solution. At high frequency with less quantity of water and nutrient applications resulted in significant reduction in leaching losses of the nutrients might have been contributed to the higher availability of nutrients for the plants and resulted in higher yield of the crop. Similar result was recorded by Himaja (2017) in sunflower with the application of 100% RDF through fertigation at weekly interval. Similar findings found by Sanju (2013) and Vasu (2011).

The data is presented in Table 2. Significantly the highest stalk yield was recorded with treatment T_7 (N and K at 4 days interval) (7652 kg ha^{-1}) which was on par with T_8 (N and K at 8 days interval) (7104 kg ha^{-1}). The stalk yield realized with T_8 was found to be at par with T_2 (manual application of N and K fertilizers + drip irrigation) (6201 kg ha^{-1}) and T_3 (N at 4 days interval) (5922 kg ha^{-1}). The lowest stalk yield was observed with T_1 (control) (3403 kg ha^{-1}). N and K at 4 days interval (T_7) has recorded 23.4% higher stalk yield compared to manual application of N and K fertilizers + drip irrigation (T_2). Manual application of N and K fertilizers + drip irrigation (T_2) has recorded 27.8% higher stalk yield compared to manual application of N and K fertilizers + furrow irrigation (T_9).

It is noticed that the highest stalk yield was recorded with application of 100% RD of N and K through fertigation over manual soil application method. This may be due to production of more foliage, uptake of more nutrients and conversion of more biomass in term of dry matter production. Optimum quantity of water and nutrient application might have reduced leaching losses of nutrients significantly and it contributes to higher availability of nutrients for the plant which resulted in higher stalk yield of the crop. Similar results were found by Himaja (2017) in sunflower at 100% RD NPK through fertigation, Soni *et al.* (2017) and Sekhar (2014).

The data is presented in Table 2. It revealed that HI was not significantly influenced by fertigation treatments. Control (T_1) and T_9 (manual application of N and K fertilizers + furrow irrigation) has recorded the highest (27.0) and the lowest was recorded under T_3 (only N at 4 days interval) (25.1). Similar result was recorded in sunflower by Himaja (2017), Sekhar (2014) and Sanju (2013).

Based on the results obtained in the present investigation, it is concluded that the sunflower crop grown with drip fertigation at 100% RD of N and K (75 kg N - 30 kg K_2O ha^{-1}) at 4 days interval from 16 to 88 DAS through Urea and Potassium Sulphate respectively, during *rabi* under Hyderabad semi arid conditions realized better yield attributes, higher seed yield (2623 kg ha^{-1}) and stalk yield (7652 kg ha^{-1}).

Table 1. Yield attributes of *rabi* sunflower as influenced by N and K fertigation schedules

Treatments	Head diameter (cm)	No. of filled seeds	Test weight (g)	Seed yield (g plant^{-1})
T_1 - Control (N_0K_0)	11.8	487	46.1	18.9
T_2 - Manual application of N and K + drip	16.2	833	68.3	32.4
T_3 - Fertigation of N at 4 days interval	14.7	806	63.7	29.7
T_4 - Fertigation of N at 8 days interval	14.5	800	60.3	27.3
T_5 - Fertigation of K at 4 days interval	13.7	771	62.2	24.9
T_6 - Fertigation of K at 8 days interval	13.5	743	59.5	24.0
T_7 - Fertigation of N and K at 4 days interval	18.6	1014	80.3	39.3
T_8 - Fertigation of N and K at 8 days interval	18.2	946	76.6	36.5
T_9 - Manual application of N and K + furrow	14.0	670	58.3	26.9
SEm ±	0.72	42.7	2.50	1.48
CD (p=0.05)	2.17	129.3	7.80	4.48

Table 2 . Yield (Kg ha^{-1}) and harvest index (%) of *rabi* sunflower as influenced by N and K fertigation schedules.

Treatments	Yield		
	Seed yield(kg ha^{-1})	Stalk yield(kg ha^{-1})	HI(%)
T_1 - Control (N_0K_0)	1262	3403	27.0
T_2 - Manual application of N and K + drip	2158	6201	25.8
T_3 - Fertigation of N at 4 days interval	1983	5922	25.1
T_4 - Fertigation of N at 8 days interval	1918	5634	25.4
T_5 - Fertigation of K at 4 days interval	1662	4617	26.5
T_6 - Fertigation of K at 8 days interval	1602	4575	25.9
T_7 - Fertigation of N and K at 4 days interval	2623	7652	25.5
T_8 - Fertigation of N and K at 8 days interval	2437	7104	25.5
T_9 - Manual application of N and K + furrow	1795	4853	27.0
SEm ±	98.9	424.7	1.99
CD (p=0.05)	299.1	1284.3	NS

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Research Note
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VARIABILITY, CORRELATION AND PATH ANALYSIS FOR SEED YIELD AND ITS COMPONENT TRAITS IN BIDI TOBACCO *(Nicotiana tabacum L)*

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Due to the growing awareness of alleged health risk factors associated with tobaccos conventional uses, research efforts need to be geared up for alternative uses of tobacco. Patel *et al.,* 1985 reported that it is possible to make use of tobacco crop for the production of several agro based chemicals. Apart from these, tobacco plant also produces oil rich seeds. Tobacco plays a prominent role in the Indian economy. Tobacco seed oil is reported to be used in certain pharmaceutical preparations, in alkyd resins and in soap manufacture. It is also used as edible oil after suitable refining in Greece, Bulgaria and some other countries (Somayajulu and Murti, 1963).

Medicinally it is used as a sedative, diuretic, expectorant, discutient, and internally only as an emetic, when all other emetics fail. The leaves in combination with the leaves of belladonna or stramonium make an excellent application for obstinate ulcers, painful tremors and spasmodic affections. The tobacco leaf juice cures facial neuralgia if rubbed along the tracks of the affected nerve. Externally nicotine is an antiseptic. Tobacco leaves put on skin inflammations to help soothe and relieve pain. Tobacco leaves could also be placed in the mouth to alleviate pain from toothaches. Nicotine in the tobacco would also help relieve pain as well as help draw out the poison and heal the snake wound. After the poison had been sucked out, chewed leaves could be applied to cuts or bound on the bite with a bandage. It has been established that tobacco seed oil is free from toxic substances. Any crop improvement programme mainly leans on the magnitude of genetic variability in the available germplasm and the extent to which it is heritable.

The information on the association of different yield components and their contribution to yield will largely benefit the breeder to evolve high yielding and stable varieties. Information on variability and correlation in tobacco, particularly for seed yield and their related traits is lacking. Therefore, an attempt was made in the present investigation to assess the variability and path analysis of 5 characters pertaining to seed yield and its components in tobacco.

Thirty genotypes of tobacco comprising local collections, established varieties and advanced breeding lines were used as experimental material. The experiment was conducted in a randomized block design with three replications at Regional Agricultural research station, Nandyal, Acharya N.G.Ranga Agricultural University, Andhra Pradesh during *Kharif* 2013. Each plot size (6.75 m x 1.5 m) consisted of a single row of ten plants with inter and intra row spacing of 75cm and 75 cm, respectively. Other agronomic practices were administered like normal tobacco crop avoiding topping and de-suckering. Five plants were selected in each genotype from each replication and observations were recorded on plant height, capsules weight per plant, individual capsule weight and seed yield per plant. Data on days to flowering was taken on plot basis, when 50% of the plants had at least one flower opened. Mean values were used for statistical analysis. The analysis of variance was carried out as suggested by Panse and Sukhatme (1961). Standard statistical procedures were followed for calculating genetic constants, phenotypic and genotypic co efficient of variation (Burton, 1952), heritability in broad sense (Burton and De vane, 1953) and genetic advance (Johansson *et al.*, 1955 a). The genotypic and phenotypic correlation co-efficient were calculated as outlined by Johnson *et al.*, (1955 b) whereas path analysis was done following the method of Dewey and Lu (1959).

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The analysis of variance showed highly significant differences among genotypes for all the characters indicating inherent variability in the materials taken up for the study. A wide range of variation was observed for all the traits (Table 1). In the present investigation, the estimate of Phenotypic coefficient of variation (PCV) was higher than the Genotypic coefficient of variation (GCV) for all the characters studied. It is obvious because PCV includes variability due to genotype and environment. The genotypic coefficient of variation (GCV) ranged from 11.92 % in days to 50% flowering to 34.56% in seed yield per plant. High genotypic coefficient of variation (GCV) for seed yield per plant and capsules weight per plant suggested considerable genetic variability for these traits in the material studied. It was further supported relatively by higher heritability percentage for these characters. Characters like days to 50% flowering, plant height, individual capsule weight showed low genotype coefficient of variation.

Table 1. Anova for different characters

Characters	Mean squares		
	Replication	Genotype	error
Days to 50% flowering	69.79	248.13	8.52
Plant height (cm)	512.24	1496.92	40.50
Capsule weight/plant (g)	136.15	1214.21	12.42
Individual capsule weight (mg)	16.72	3186.95	67.24
Seed yield/ plant (g)	53.20	531.24	9.15

The estimates of heritability are useful to plant breeders as they provide fundamental basis for selection on phenotypic performance. The heritability estimates obtained were high for all the traits under study.

The highest heritability was exhibited by capsules weight per plant followed by seed yield per plant and individual capsule weight. Heritability estimates along with genetic advance are usually more useful than heritability alone in predicting the resultant effect of selecting the best individuals (Johnson *et al.*, 1955a). In the present study, estimates of genetic advance expressed as percentage of mean were high capsules weight per plant followed by seed yield/ plant: moderate for individual capsule weight, plant height and days to 50% flowering (Table:2).

Table 2. Estimates of variances and other genetic parameters for five characters in tobacco

Characters	Mean	Range		Phenotypic variance	Genotypic variance	PCV (%)	GCV(%)	h^2_b(%)	Genetic advance as % of mean
		Min.	Max.						
Days to 50% flowering	72.74	64.20	94.10	84.19	75.27	10.44	11.92	94.18	22.48
Plant height (cm)	142.72	106.73	184.27	536.04	514.74	17.26	15.67	91.43	28.05
Capsule weight/plant (g)	61.57	32.57	114.20	414.95	367.93	32.81	37.37	98.23	68.72
Individual capsule weight (mg)	206.83	119.35	268.17	1073.01	1126.17	18.18	18.95	94.66	36.80
Seed yield/ plant (g)	38.41	18.14	66.10	187.48	188.13	36.43	34.56	97.10	64.56

PCV= phenotypic coefficient of variation, GCV= Genotypic coefficient of variation, h^2_b = heritability in broad sense

The estimation of genetic parameters revealed that capsules weight per plant and seed yield per plant had high heritability as well as high genetic advance. These characters also expressed high genotypic coefficient of variation. It may, therefore, be inferred that additive genes were largely responsible for variation among genotypes for these traits (Panse, 1957). Hence, selection in early segregating generation for such highly heritable characters is expected to give good results. The characters like individual capsule weight, plant height and days to 50% flowering had high heritability and moderate genetic advance and therefore, there is a scope for improvement through straight selection. Very low value of genetic coefficient of variation for days to 50% flowering indicated the predominance of non-additive genes. Under such situation, recurrent selection (Comstock et al., 1949) would prove useful for improving these traits.

Genotypic and phenotypic correlation coefficient between seed yield and related components are presented in (Table 3). The correlation at genotypic and phenotypic level, in general, showed the same trend. Genotypic correlations were generally higher than the corresponding phenotypic correlations. The low phenotypic correlation could result due to the modifying effect of environment on the association of characters at the genic level. Seed yield per plant exhibited highly significant and positive correlation with capsules weight per plant and individual capsule weight both at phenotypic and genotypic levels, whereas it's correlation with days to 50% flowering and plant height was negative. Days to 50% flowering had significant and positive correlation with plant height. The capsules weight per plant with individual capsule weight and individual capsule weight with seed yield showed significant and positive interrelationship.

Table 3: Genotypic (G) and Phenotypic (P) correlation coefficients for five characters in tobacco

Characters		Plant height	Capsule weight/plant	Individual capsule weight	Seed yield/plant
Days to 50% flowering	G	0.412**	-0.244	-0.146	-0.239
	P	0.356**	-0.238	-0.136	0.244
Plant height (cm)	G		-0.218	-0.287	-0.269
	P		-0.182	-0.257	-0.233
Capsule weight/plant (g)	G			0.426*	0.979**
	P			0.417*	0.962**
Individual capsule weight (mg)	G				0.479**
	P				0.454**

* and ** denote significant at 5 and 1 percent levels, respectively.

Path coefficient analysis were useful for sorting out the correlations into direct and indirect effects for different characters on seed yield. The path analysis (Table 4) indicated that the capsules weight per plant had the maximum positive direct effect on seed yield followed by individual capsule weight. The same two characters also exhibited highly significant positive correlations with seed yield per plant. Therefore, it would be rewarding to lay more emphasis on capsules weight per plant and individual capsule weight per plant and individual capsule weight in selection programme for improving the seed yield in tobacco. Days to50% flowering had small negative direct effect on seed yield and their indirect effects though other variables were either negative or negligible (Table 4). Plant height had positive direct effect but their indirect effects through other characters were negative.

Table 4. Direct and Indirect effects of four component characters on seed yield in tobacco

Characters	Days to 50% flowering	Plant height (cm)	Capsule weight/ plant (g)	Individual capsule weight (mg)	Genotypic correlations with seed yield/plant
Days to 50% flowering	-0.0551	-0.0242	0.0117	0.0078	-0.2393
Plant height (cm)	0.0272	0.0511	-0.0134	-0.0139	-0.2486
Capsule weight/plant (g)	-0.2047	-0.2346	0.9429	0.4022	0.9768**
Individual capsule weight (mg)	-0.0216	-0.0441	0.0683	0.1629	0.4944**

** denote significant at 1 per cent level

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Research Note
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DETERMINING STORAGE POTENTIAL OF NATURALLY AGED SEEDS OF PADDY VARIETIES

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Even though rice seed is a good storer, loss in seed quality during storage frequently takes place especially in humid tropical and subtropical areas. Seed deterioration is a natural process which is expressed as loss of quality, viability and vigour during ageing or adverse environmental conditions. The rate of deterioration is however influenced by seed moisture content and temperature of the storage. Many physical and biochemical manifestations of seed deterioration have been reported by Jatoi *et al.* (2004). Most widely accepted single criteria of seed deterioration is reduced germination. In order to preserve the planting value of seed, information on storage potential of important varieties is essential. Manipulation of storage environment and usage of suitable packaging material has to be recommended for certain varieties having relatively poor storability. As information available regarding the storage response and relative storability of cereal crops in general and relative storability of different paddy varieties in particular is meagre, present study was undertaken to estimate seed storage potential and relative storability of naturally aged seeds of seven paddy varieties viz., Tellahamsa, RNR-15048, WGL-347, KNM-118, JGL-11118, JGL-11470 and JGL-18047 of which six are recently released by Professor Jayashankar Telangana State Agricultural University except Tellahamsa.

Laboratory experiment was conducted to study changes during the seed storage of paddy varieties under natural ageing with different packaging materials at the Department of Seed Science and Technology, Seed Research and Technology Centre, PJTSAU, Rajendranagar, Hyderabad. The freshly harvested seeds of above varieties was used which had initial germination percentage above Indian Minimum Seed Certification Standards (IMSCS) and were stored under ambient conditions in different packaging materials (Gunny bag, polylined gunny bag and polypropylene woven bag). The data on seed germination and seedling vigour index were recorded bimonthly for seeds stored in all three packaging materials upto 6 months. Seed germination test was conducted as per ISTA rules (Anon, 2016) and seedling vigour index II was estimated as per the procedure given by Abdul-Baki and Anderson (1973). Three factorial completely randomized design was followed (Sahu and Das,2014) The varieties (seven), packing materials (three) and storage periods (three bimonthly intervals) were considered as factor 1,2 and 3 respectively. The analysis of variance revealed significant effect of treatments.

Among the treatments, the highest germination was recorded in KNM-118/gunny bag (97%) followed by RNR-15048/polypropylene bag(96%), WGL-347/ gunny bag (96%) , JGL-11118/ polypropylene bag (96%), KNM 118/ polylined gunny bag, RNR-15048/gunny bag (95%) and WGL-347/ polypropylene (95%) which came under one significant group and were on par with each other at 2 months after storage. Significantly low germination was recorded in Tellahamsa with all the three packaging materials showing relatively poor storability in terms of germination (Table1).

After 6 months of storage, the treatment KNM-118 / polylined gunny bag recorded significantly high germination (94%) over all other treatments (Table 1). The other varieties in different packing materials recorded more than 80% germination. At this storage interval, the germination of Tellahamsa in all the three packaging materials was below the IMSCS. The variety JGL-18047 recorded (70%) germination in polypropylene bag, which was also below IMSCS.

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Table 1. Effect of packaging material and storage period on seed germination (%) of paddy varieties

Varieties	2nd month				4th month				6th month			
	P1	P2	P3	Mean	P1	P2	P3	Mean	P1	P2	P3	Mean
Tellahamsa	87(68)*	87(68)	86(68)	**85(68)**	84(66)	76(60)	82(64)	**81(63)**	76(60)	63(52)	61(51)	**67(54)**
RNR-15048	95(77)	92(73)	96(78)	**94(76)**	88(69)	88(69)	96(78)	**91(72)**	85(67)	88(69)	86(68)	**86(68)**
WGL-347	96(78)	94(75)	95(77)	**95(77)**	93(74)	89(70)	93(74)	**91(73)**	84(66)	87(68)	86(68)	**86(67)**
KNM-118	97(80)	96(78)	93(74)	**95(77)**	94(75)	94(75)	93(74)	**94(75)**	93(74)	94(75)	91(72)	**93(74)**
JGL-11118	92(73)	90(71)	96(78)	**93(74)**	94(75)	88(69)	92(73)	**91(72)**	85(67)	81(64)	88(69)	**85(67)**
JGL-11470	92(73)	94(75)	92(73)	**93(74)**	85(67)	88(69)	94(75)	**89(70)**	90(71)	81(64)	88(69)	**86(68)**
JGL-18047	94(75)	97(80)	88(69)	**93(75)**	88(69)	87(68)	92(73)	**89(70)**	88(69)	81(64)	70(56)	**80(63)**
Mean	**93(75)**	**93(74)**	**93(74)**	**93(74)**	**89(71)**	**87(69)**	**92(73)**	**89(71)**	**87(68)**	**82(64)**	**83(65)**	**83(66)**
C.V (%)		**3.33**		**4.12**					**4.98**			

	Varieties x packaging material	Varieties x storage period	Packaging material x storage period	Varieties x packaging material x storage periods
SEm(±)	1.039	1.039	0.68	1.799
CD (0.05)	2.907	2.907	1.903	5.035

( )*- arcsine transformed values
P1 : Gunny bag, P2 : Polylined gunny bag, P3:Polypropylene woven bag

The overall interaction effects of packaging material, storage period and varieties on germination of paddy were given in (Table 2). The germination percentage decreased significantly from 2nd (93%) to 6th months (83%) of storage. Among the packaging materials, polypropylene bag recorded the highest germination (90%) which was on par with gunny bag (89%) compared to polylined gunny bag (87%).The varietal response with respect to germination showed that KNM-118 maintained significantly higher germination (94%) among all other varieties. Except Tellahamsa, all other varieties recorded germination above IMSCS.

The treatment KNM-118/polypropylene recorded the highest vigour index –II (17843) followed by KNM 118/gunny bag (16977) and KNM 118/ Polylined gunny bag(16225) among other treatments at 2nd month of storage. Lowest vigour was reported in RNR-15048/gunny bag (6639). At 6th month of storage, KNM-118 in all three packaging materials recorded higher values than other treatments. Lowest was recorded in RNR-15048/gunny bag (6096) and RNR-15048/poly propylene bag (6043) (Table 3). Among packaging material polypropylene bag had higher vigour index (10588) followed by gunny bag (10156) compared to polylined gunny bag. Irrespective of packaging material and storage period KNM-118 recorded (16764) significantly highest vigour index II among others (Table 4). Seed deterioration is loss of seed quality, viability and vigour due to effect of adverse environmental factors as given by Kapoor *et al.* (2010).

From the data obtained from natural ageing of paddy varieties, KNM-118 was found superior with respect to germination and seedling vigour whereas Tellahamsa recorded low germination below IMSCS at six months after storage. The gunny bag was equally good with that of polypropylene bag up to 6 months of storage during post rainy season under Hyderabad conditions. The varieties with good early seedling vigour can better establish under different growing situations as reported by Krishnasamy and Seshu (1989). The varieties with poor storability need special packaging material/storage conditions to safeguard the seed germination and vigour, however the studies on storability of varieties has to be continued further till the germination falls below IMSCS in all the treatments.

Table 2. Seed germination (%) as influenced by interaction among the packaging material, storage period and varieties in paddy

Storage period	Germination	Packaging materials	Germination	Varieties	Germination
2nd month	93(74)*	Gunny bag	89(70)	Tellahamsa	78 (62)
4th month	89(71)	Polylined gunny bag	87(68)	RNR-15048	90 (71)
6th month	83(66)	Polypropylene bag	90(71)	WGL-347	91 (72)
				KNM-118	94 (74)
				JGL-11118	89 (70)
				JGL-11470	90 (71)
				JGL-18047	87 (68)
Mean	**88(69)**		**88(69)**		**88(69)**
SEM (±)	**0.393**		**0.393**		**0.6**
CD (0.05)	**1.1**		**1.099**		**1.678**

( )*- arcsine transformed values

Table 3. Effect of packaging materials and storage period on seedling vigour index II of paddy varieties

Varieties	2nd month				4th month				6th month			
	P1	P2	P3	Mean	P1	P2	P3	Mean	P1	P2	P3	Mean
Tellahamsa	12967	12342	13088	**12799**	11921	10387	12062	**11457**	10135	8289	7992	**8805**
RNR-15048	6639	7074	7771	**7161**	6892	6655	7765	**7104**	6096	6463	6043	**6201**
WGL-347	9363	8962	10090	**9472**	9040	8548	10191	**9260**	7660	7827	8080	**7856**
KNM-118	16977	16225	17843	**17015**	17089	16773	16860	**16907**	16070	16280	16801	**16369**
JGL-11118	9161	9116	10477	**9585**	9273	7570	9443	**8762**	7945	7965	7940	**7950**
JGL-11470	7447	7512	7096	**7952**	6999	7369	7869	**7412**	6782	6376	6775	**6644**
JGL-18047	13233	12613	13183	**13010**	11413	11583	14814	**12603**	10448	10189	10170	**10269**
Mean	**10827**	**10549**	**11364**	**10900**	**10375**	**9841**	**11286**	**10501**	**9305**	**9056**	**9114**	**9158**
C.V (%)		**5.47**		**4.32**		**4.29**						

	Varieties x packaging material	Varieties x storage period	Packaging material x storage period	Varieties x packaging material x storage periods
SEm(±)	191.82	191.82	125.57	321.31
CD (0.05)	536.84	536.84	351.44	899.38

P1 : Gunny bag, P2 : Polylined gunny bag, P3:Polypropylene woven bag

Table 4. Seedling vigour index - II as influenced by interaction among packaging material, storage period and varieties in paddy

Storage period	Vigour index - II	Packaging materials	Vigour index - II	Varieties	Vigour index - II
2nd month	10900	Gunny bag	10156	Tellahamsa	11020
4th month	10501	Polylined gunny bag	9815	RNR-15048	6822
6th month	9158	Polypropylene bag	10588	WGL-347	8862
				KNM-118	16764
				JGL-11118	8766
				JGL-11470	7336
				JGL-18047	11961
Mean	10187		10122		10569
SEm (±)	70.118		70.118		107.1
CD (0.05)	196.262		196.262		299.79

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EFFECT OF N AND K FERTIGATION LEVELS ON TOTAL FRUIT YIELD, YIELD ATTRIBUTES AND WATER PRODUCTIVITY OF PAPRIKA (*Capsicum annuum.L*)

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Fertigation is a frontier technology, which permits application of various nutrients and fertilizer formulations directly at the site of active roots in desired concentration, time and thus improves the nutrient use efficiency and the yield of crops. Paprika (*Capsicum annuum L.*) is a less pungent widely used chilli variety and is an important vegetable cum condiment and is used as an ingredient in a broad variety of dishes throughout the world. There is a need to study the vital aspects of fertigtion for different crops involving the sources of fertilizers, rate of fertilizers, frequency of fertigation, methods of fertilizer application through soil, drip irrigation, combination of soil and irrigation water and combination of nutrients for fertigation etc.

The present experiment on paprika was conducted during *rabi* 2014-2015 at College Farm, Water Technology Centre, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad. The soil of the experimental site was sandy loam in texture with a pH of 8.2, electrical conductivity of 0.22 dS m^{-1}, high in organic carbon (1.15 %), low in available nitrogen (149 kg ha^{-1}), medium in available phosphorus (50.2 kg P ha^{-1}) and high in available potassium (346 kg K ha^{-1}). The water source for irrigation was from an open well. The irrigation water used in the experiment was alkaline (pH=7.89) and categorized under the Class II (C_3S_1). The experiment was laid out in a simple randomized block design with three replications. The treatments were eleven, *viz.*, soil application of 100 % N and K_2O with drip irrigation (T_1) and with furrow irrigation (T_2); drip fertigation of 75 % N + 75 %, 100 % and 125 % K_2O (T_3, T_4, T_5, respectively); drip fertigation of 100 % N + 75 %, 100 % and 125 % K_2O(T_6, T_7, T_8 respectively); drip fertigation of 125 % N + 75 %, 100 % and 125 % K_2O (T_9,T_{10},T_{11} respectively).

In drip system, the laterals of 16 mm diameter were laid at 1.2 m apart with spacing of 0.4 m distance between two inline emitters. Control valves were fixed separately to each treatment plot to facilitate controlling the water flow as per the treatments in the experiment. Drip Irrigation scheduling was done as per the treatments. Scheduling of irrigation for treatments T_1 to T_{11} (except T2) were fixed for once in 2 days based on daily evaporation data recorded from USWB class 'A' pan evaporimeter in agro-meteorological station, ARI Farm, Rajendranagar, Hyderabad. The irrigation duration was based on the number of laterals, emitter spacing and emitter discharge for a given design area. In surface furrow irrigation treatment (T_2), furrows were made in between the two paired rows at 80 cm/40 cm apart. Surface irrigation was scheduled at 1.0 IW/CPE ratio where in 60 mm depth of irrigation water was applied whenever CPE ratio reached to 87 mm during the experiment.

The Chilli paprika crop (variety -Agnirekha, Syngenta company) was grown in nursery and 36 days aged seedlings were transplanted manually. The crop was supplied with a common basal dose of 100 kg P_2O_5 ha^{-1} through single superphosphate. The nitrogen was applied through urea. The potassium was applied through potassium nitrate in fertigation treatments (T_3 to T_{11}) and through muriate potash (MOP) in soil application treatments (T_1 and T_2). The amount of nitrogen supplied through potassium nitrate was considered while calculating the quantity of urea dose for fertigation. The 100% recommended dose of N and K was 250 and 150 kg N and K_2O ha^{-1} respectively. In fertigation treatments, the N and K were applied in 38 splits starting from 12 DAT to 144 DAT @ twice in a week. Soil application of fertilizers in T_1 and T_2, the N and K applied was in three equal splits at 12, 35 and 60 DAT. Need based plant

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protection measures were taken during nursery and main crop growth periods. The amount of water used for irrigating the crop was measured by using a water meter. The amount of total irrigation water applied was 6381 m^3 and 7483 m^3 in drip and furrow irrigated treatments (T_2), respectively. The effective rainfall received during the crop growth period was 53.7 mm and 72.3 mm in drip and furrow irrigated treatments, respectively.

In total, six times the paprika fruits were harvested at 57, 70, 90, 117, 138 and 160 DAT. The first five pickings consisted of green fruits and the last picking comprised of red fruits. During each picking, along with fresh fruit yield, the yield attributes like fruit length, girth, number of fruits per plant and one fruit weight were recorded. The water productivity was computed and expressed as fresh fruit yield in kg per cubic meter of water used.

The data on mean of yield attributes (fruit length, width and fruit weight (mean of six pickings) and total number of fruits (sum of six pickings), total fresh fruit yield (sum of six pickings) and water productivity are presented in Table 1. The mean fruit length of paprika (mean of six pickings) ranged from 2.71 to 3.93 cm. The highest fruit length was noticed with fertigation of 125 % N and K_2O (T_{11}) which was on par with 125% N with different K_2O levels (T_{10} and T_9) and 100% N with 125% K_2O (T_8) and was significantly higher than other treatments. In general higher fruit length was noticed with increase in N levels from 75% to 125%. Within each N level, with increase in K levels from 75% to 125% there was increase in fruit length. However, within each N level, K levels were statistically on par to T_6, T_7. Soil application of 100% N and K and irrigated by drip (T2) was observed to be on par with 75% N + 125% K_2O (T_3) and was significantly lower than other fertigated treatments and significantly higher than soil application of 100% N and K and irrigated by furrow method (T_2). Fertigation of 100% N and K (T_7) (3.68 cm) resulted in 20.26 % increase in length of fruits compared to soil application of same levels of N and K under drip irrigation (T_1) (3.06 cm). There was 12.91% increase in length of fruits by drip irrigation at 100% N and K applied to soil (T_1) (3.06 cm) compared to furrow irrigation (T_2) at same N and K levels (2.71 cm). This increase in fruit size could well be attributed to the increased rate of photosynthesis which could have further led to the partitioning of assimilates. Many characters of the fruit like cell size, lactiferous canals, intercellular spaces, etc., in different tissues of the fruit contribute to the increase in length. (Malik *et al.* 2011, Gupta *et al.* 2010).

The mean fruit girth of paprika (mean of six pickings) ranged from 2.22 to 4.60 cm. The highest fruit girth was noticed with fertigation of 125 % N and K_2O (T_{11}) which was on par with 125% N with 100 K_2O levels (T_{10}) and was significantly higher than other treatments. Similar to fruit girth, in general higher fruit girth was noticed with increase in N levels from 75% to 125%. Within each N level, with increase in K levels from 75% to 125% there was increase in fruit girth. Within each N level, 75% and 100% K_2O levels were statistically on par to each other. Soil application of 100% N and K and irrigated by drip (T_2) was on par with 75% N + 125% K_2O (T_3) and was significantly lower than other fertigated treatments and significantly higher than soil application of 100% N and K and irrigated by furrow method (T_2). Fertigation of 100% N and K (T_7) (3.37 cm) resulted in 35.4 % increase in girth of fruits compared to soil application of same levels of N and K under drip irrigation (T_1) (2.49 cm). There was 12.16% increase in girth of fruits by drip irrigation at 100% N and K applied to soil (T_1) (2.49 cm) compared to furrow irrigation (T_2) at same N and K_2O levels (2.22 cm). In the present study, adequate supply of nutrients, like 125 per cent of N and K could have increased the above said parameters specially combined with fertigation. The results are in agreement with that of Gupta *et al.* (2010).

The mean fruit weight (mean of six pickings) ranged from 4.78 to 6.88 g $fruit^{-1}$. The highest fruit weight was noticed in 125 % N + 100 % K_2O (T_{10}) application which was on par with 125% N + 75% K_2O (T_9), 125% N + 125% K_2O (T_{11}) and 100% N + 125% K_2O (T_8) and was significantly higher over other treatments. In general higher mean weight $fruit^{-1}$ was noticed with increase in N levels from 75% to 125%. Within each N level, with increase in K levels from 75% to 125% there was increase in fruit weight. Within each N level, 125% and 100% K_2O levels were statistically on par to each other and significantly higher over 75% K_2O level. Significantly the lowest weight of fruit was recorded in 100% N and K application to soil and furrow irrigation (T_2). Fertigation of 100% N and K (T_7) (6.48 g $fruit^{-1}$) resulted in 18.4

% increase in weight of fruits compared to soil application of same levels of N and K and drip irrigated (T_1) (5.47 g fruit^{-1}). There was 14.4 % increase in weight of fruits by drip irrigation at 100% N and K applied to soil (T_1) compared to furrow irrigation (T_2) at same N and K levels (4.78 g fruit^{-1}). Many characters of the fruit like cell size, lactiferous canals, intercellular spaces, etc., in different tissues of the fruit contribute and adequate supply of nutrients to the increase in weight (Malik *et al.*, 2011). Similar results are also reported by Nissar Naeem *et al.* (2002).

The total number of fruits (sum of six pickings) ranged from 10.39 to 21.27 plant^{-1}. The highest no. of fruits were recorded in fertigation of 125% N + 125% K (T_{11}) which was on par with 125% N with 100% K_2O (T_{10}) and 75% K_2O (T_9) and was significantly higher over other treatments. Like the fruit length and width, in general higher number of fruits per plant were noticed with increase in N levels from 75% to 125%. At 100% and 75% N levels, with increase in K levels from 75% to 125% there was significant increase in number of fruits per plant. Whereas, at 125% N levesl, all the K_2O levels were statistically on par with each other. The lowest fruit no. plant^{-1} was recorded in 100% N and K application to soil and furrow irrigation (T_2) which was significantly lower than all other treatments. Fertigation of 100% N and K (T_7) (18.62) resulted in 35.41 % increase in no. of fruits plant^{-1} compared to soil application of same levels of N and K under drip irrigation (T_1) (13.75). There was 32.3% increase in no. of fruits plant^{-1} by drip irrigation with 100% N and K_2O applied to soil compared to furrow irrigation (T_2) at same N and K_2O levels (10.39) applied to soil. Continuous availability of moisture and nutrients in root zone of fertigated treatments might have improved the availability of native and applied nutrients. This might have accelerated the synthesis of carbohydrates and its better translocation from sink to source that might have led to an improvement in yield and yield related attributes. The number of fruits plant $^{-1}$ increased gradually with the increase of nitrogen dose was reported by several scientists (Bhuvaneswari *et al.* 2013, Malik *et al.*, 2011).

The total fresh fruit yield ranged from 9611 to 22,076 kg ha^{-1}. The highest total fresh fruit yield was recorded in 125 % N + 75% K (T_9) which was on par with 125 % N + 125 % K (T_{11}) (20,822 kg ha^{-1}) and 125 % N + 100 % K (T_{10}) (20,721 kg ha^{-1}). The lowest yield was recorded in soil application of 100 % N and K and furrow irrigation (T_2) (9611) which was on par with fertigation with 75% N + 125 % K (T_5) (12584 kg ha^{-1}). In general within each level of N, increase in K level from 75 % to 100 or 125 % decrease in total yield trend was shown indicating that 75 % K level is sufficient for the paprika crop under the present experimental soil conditions. As the initial soil available potassium status was high (346 kg K ha^{-1}), significant response to higher K_2O levels beyond 75% of recommended dose was not noticed in this experiment. However, among N levels, the response was positive up to 125% N. Application of 100 % N and K applied through fertigation (T_7) resulted in 17% increase in yield compared to same level of N and K applied through soil application and irrigation by drip (T_1). The same level of soil application of 100 % N and K and drip irrigation treatment (T_1) has recorded 56 % higher total fresh paprika fruit yield (15,005 kg ha^{-1}) compared to soil application of same dose of N and K but irrigation by furrow method (T_2) (9611 kg ha^{-1}). Application of nutrients by fertigation at more frequent intervals during different growth stages leads to its availability in the vicinity of the root zone resulting in more efficient utilization of applied nutrients than soil application method. Fertigation leads to precise application of nutrients to the restricted volume of soil where the active roots will be concentrated and hence will be available to plants fully. (Malik *et al.* 2011, Gupta *et al.* 2010).

The water productivity of paprika in the present experiment ranged from 1.28 to 3.45 kg m^{-3}. The highest water productivity was recorded by application of 125% N + 75% K (T_9) which was on par with 125% N + 125 % K (T_{11}) and (T_{10}) 125% N + 100 % K and was significantly higher over all other treatments. At 75 % and 100 % N levels (T_3 to T_8), increase in K levels from 75 % to 100 and 125 % resulted in decrease in water productivity. At 125 % N level (T_9 to T_{12}) increase in K level from 75 % to higher levels, resulted in lower water productivity, however 100 and 125 % K levels recorded on par water productivity. The lowest water productivity was recorded in soil application of 100 % N and K and irrigation by furrow method (T_2) which was 1.83 times lower than the treatment where same level of N and K was applied but irrigated by drip method (T_1).

Increase in water productivity to the 17.45 per cent was recorded by T_7 (2.76 kg m^{-3}**)** where 100 % N and K was applied through fertigation compared to same levels of N and K applied through soil application but irrigated by drip T_1 (2.35 kg m^{-3}**).** Hood (2002) described water use efficiency (WUE) in irrigated agriculture as maximizing the returns and minimizing the environmental impacts for every mega liter (ML) of water used for irrigation purposes.). Improvement in WUE was due to increase in total fruit yield due to application of 125% N, P and K was reported by Ramachandrappa *et al.* (2010). Water saving under micro-irrigation system ranging from 46.4 to 67.81 per cent over furrow irrigation method was reported by Ayare *et al.* (2012).

The study revealed that during *rabi* season, application of 125% N + 75% K2O (312.5 kg N + 112.5 kg K_2O ha^{-1}) by fertigation in 38 splits from 10 DAT to 144 DAT was found to be optimum for growing of paprika in red sandy loam soils.

Table1. Effect of N and K fertigation levels on mean fruit length, fruit girth, fruit weight (mean of six pickings), total number of fruits plant^{-1}, total fresh fruit yield (sum of six pickings) and water productivity of paprika during *rabi* 2014-2015.

S.No	Treatments	Mean fruit length (cm)	Mean fruit girth (cm)	Mean fruit weight (g)	Total no. of fruits plant^{-1}	Total fresh fruit yield (kg ha^{-1})	Water productivity (kg m^{-3})
T_1	Soil application of 100% N and K + drip irrigation	2.49	5.47	13.75	15005	2.35	3.06
T_2	Soil application of 100% N and K + furrow irrigation	2.71	2.22	4.78	10.39	9611	1.28
T_3	Fertigation of 75 % N + 75 % K	3.11	2.59	5.53	14.96	18250	2.86
T_4	Fertigation of 75 % N+ 100 % K	3.31	2.89	5.92	15.92	17188	2.69
T_5	Fertigaion of 75 % N + 125 % K	3.46	2.92	6.08	16.83	12584	1.97
T_6	Fertigation of 100 % N + 75 % K	3.59	3.25	6.12	17.43	18128	2.84
T_7	Fertigation of 100 % N+ 100 % K	3.68	3.37	6.48	18.62	17630	2.76
T_8	Fertigaion of 100 % N + 125 % K	3.77	3.99	6.60	20.44	17189	2.69
T_9	Fertigation of 125 % N + 75 % K	3.84	4.21	6.87	20.91	22076	3.45
T_{10}	Fertigation of 125 % N+ 100 % K	3.92	4.40	6.88	20.89	20721	3.24
T_{11}	Fertigaion of 125 % N + 125 % K	3.93	4.60	6.86	21.27	20822	3.26
	S.E. m +/-	0.05	0.08	0.10	0.25	1279	0.20
	CD (P=0.05)	0.16	0.22	0.30	0.75	3772	0.58

75 % N = 187.5 kg N ha^{-1}, **100% N** = 250 kg N ha^{-1},**125 % N** = 312.5 kg N ha^{-1} ,
75 % K = 112.5 kg K_2O ha^{-1}, **100% K=** 150 kg K_2O ha^{-1}, **125 % K_2O** = 187.5 kg K_2O ha^{-1}.

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Research Note
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SEASONAL INCIDENCE OF MIRID BUG (*Cyrtorhinus lividipennis* REUTER), PREDATOR OF BROWN PLANT HOPPER IN RICE ECOSYSTEM

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Rice (*Oryza sativa* L.) is the staple food crop of more than half of the world's population**.** Asia is the leader in rice production accounting for about 90 per cent of the world's production. India is the second largest producer and consumer of rice in the world after China which accounts for 21 per cent of the world's total rice production. In India, rice is grown in an area of 43.49 million hectares with an annual production of 111.01 million tonnes (India stat, 2017-18).

Rice production is adversely affected by numerous biotic and abiotic stresses. Approximately 52 per cent of the global rice production is lost annually owing to the damage caused by biotic factors, out of which 21 percent is attributed to the attack of insect pests (Yarasi *et al*., 2008). Out of many insect species, 100 species causes damage to rice crop. The stem borer, brown planthopper (BPH) and gall midge are among the important pests in Southeast Asia and China and accounted for major yield loss in South Asia (Sardesai, 2001). Damages caused by insects disturb physiology of plants and result in lower crop yield (Nasiruddin and Roy, 2012)

In most rice ecosystems, non-classical or Inundative biological control is necessary for pest management because the naturally occurring predators are sufficient for economic control in almost all the cases. A wide variety of natural enemies are available in rice fields and these natural enemies can regulate insect pest incidence in rice.

These conditions have lead to growing interest in recent times in the conservation of existing entomophages in rice cropping systems with enhanced biological attributes through conservation biological control (CBC) (Eilenberg *et al.,* 2001). Hence the present investigation was taken up to record the seasonal abundance of mirid bugs in rice ecosystem.

The present field study was conducted at Regional Agricultural Research Station (RARS), Warangal of Professor Jayashankar Telangana State Agricultural University (PJTSAU), Rajendranagar, Hyderabad, Telangana State during *Kharif*, 2015 and 2017. Paddy seedlings (BPT5204) of 25-35 days age were transplanted in an area of 10 x 6 m per replication during first week of July 2015 and 2017 at a distance of 20 x 15 cm. Recommended package of practices of PJTSAU were followed for raising the crop. The crop was kept unsprayed throughout the crop season. The experiment was replicated three times and maintained separately.

Observations of mirid bugs were taken by visual counting of the population at fortnightly intervals from 30 DAT till the harvest of paddy crop at 30, 45, 60, 75, 90, 105 and 120 DAT. The population counts were taken from 20 randomly selected hills. The data recorded on the population of mirid bugs during *Kharif*, 2015 and 2017 were pooled and data was subjected to suitable statistical analysis for presentation of results

The population of mirid bugs appeared in the crop during I fortnight of August in *Kharif,* 2015 with a low population of 1.07 bugs per hill and reached to peak population of 2.97 per hill at 90 DAT (Table 1 and Fig 1). The mean population of mirid bugs recorded during *Kharif,* 2015 was 1.75 per hill.

In *Kharif*, 2017 also low population of mirid bugs was observed (1.20 mirid bugs per hill) at 30 DAT and the mirid bugs continued to occur on the

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crop till the harvest at 120 DAT (Table 1). During this season, the population of mirid bugs ranged from 1.20 to 2.84 per hill. Maximum population of mirid bugs of 2.84 per hill were recorded at 90 DAT followed by 2.16 per hill at 105 DAT (Fig 1). Across the season the mean mirid bug population of 1.84 per hill was noticed.

The pooled mean population of mirid bugs observed during both seasons together showed that the population ranged from 1.13 per hill to 2.91 per hill (Table 1). Low population of mirid bugs of 1.13 per hill was recorded at 30 DAT followed by 1.33 per hill at 45 DAT. Maximum population of mirid bugs of 2.91 per hill was recorded at 90 DAT followed by 1.79 per hill at 75 DAT (Fig 1). The pooled mean population of the mirid bugs population recorded was 1.80 per hill.

The peak population of mirid bugs which coincided with the peak incidence of BPH and the correlation coefficients worked out between the mirid bug populations. From the results it was evident various weather parameters like minimum temperature and morning relative humidity showed highly significant effect while maximum temperature exhibited negative and significant relation with mirid bugs population. The evaporation and evening relative humidity, rainfall and sunshine hours did not show any influence on mirid bug population.

The present findings are in accordance with Prasanth (2010) who reported that spiders, mirid bugs (*Cyrtorhinus lividipennis*) and carabid predators of BPH were recorded in higher number (4.25 ±1.50 to 16.50 ± 5.07) coinciding with the corresponding months with highest incidence of BPH. Similiarly earlier workers have recorded the mirid predators to be active from first fortnight of October till the harvest (0.90 to 4.00/hill) of the crop (Vijaykumar and Patil, 2006). The present results clearly showed the prevalence of the mirid bugs in rice throughout the crop season. Hence reducing the spraying of insecticides during the peak occurance of mirid bugs (90 DAT) will help in the conservation of mirid bugs and better management of BPH in the rice fields.

Table 1. Seasonal incidence of mirid bugs, *Cyrtorhinus lividipennis* on rice crop during *Kharif,* 2015 and 2017

Population of mirid bugs (No/ hill)					
Days after transplantation (DAT)	**Standard Weeks**	***Kharif*, 2015**	**Standard Weeks**	***Kharif*, 2017**	**Pooled data (*Kharif*, 2015 and 2017)**
30	33 August I FN	1.07	34 August II FN	1.20	1.13
45	35 August II FN	1.20	36 September I FN	1.45	1.33
60	37 September I FN	1.78	38 September II FN	1.78	1.78
75	39 September II FN	1.53	40 October I FN	2.05	1.79
90	41 October I FN	2.97	43 October II FN	2.84	2.91
105	44 October II FN	2.05	45 November I FN	2.16	2.11
120	46 November I FN	1.64	47 November II FN	1.43	1.54
Mean		1.75		1.84	1.80

FN- Fortnight

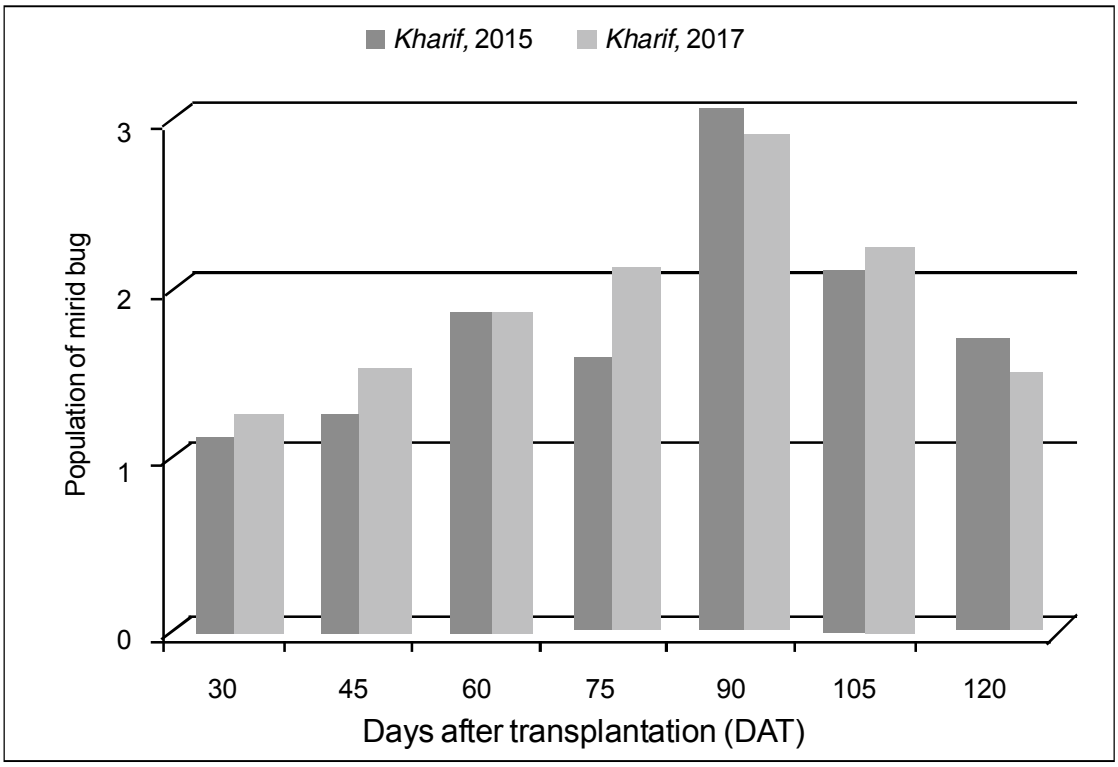

Fig 1 Seasonal incidence of mirid bugs on rice crop during *Kharif,* 2015 and 2017

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SUPPLY CHAIN OF SELECTED FRUITS IN HYDERABAD AND SOURCES OF FINANCE TO THE SUPPLY CHAIN PARTNERS

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The flow of funds to and among the various links within supply chain comprises what is known as supply chain finance. Stated another way, it is any or all of the financial services, flowing to and/or through a value chain to address the needs and constraints of those involved in that chain to access finance. (Miller and Jones, 2010).

Agricultural supply chains in India are much unorganized where each partner in the supply chain suffers from inefficiencies due to the lack of coordination, no proper infrastructure like warehouses and difficulties in access to finance and non-remunerative prices for the farmers. The marketing channels are clogged with middlemen and there is no transparent pricing mechanism in markets.

Horticulture also throws varied challenges and the marketing system is highly inefficient in case of fruits. The market infrastructure is better developed for food grains but fruits and vegetables markets are not that well developed and are congested and unhygienic. Lack of warehousing and cold storage facilities prevents the farmer and the traders to get remunerative prices for their produce. Presence of large number of intermediaries makes the farmer to greatly rely on intermediaries. Marketing infrastructure for grading, standardisation at the mandis is another challenging factor. Lesser control on product safety and quality across the supply chain because of manual handling leads to higher degradation of the produce. Lack of transparency in pricing and heavy fluctuations in the prices in the mandis challenges the farmers from getting proper prices for their efforts and fresh produce. High wastage along the supply chain and losses in transportation also reduce the income of supply chain partners. These features make the marketing system of fruits to differ from other agricultural commodities, particularly in providing time, form and space utilities. (Agriculture Today, 2016)

Various supply chains are adopted to see that the fruits reach the final consumer. This paper tries to throw light on the supply chain networks in operation for fruits, sources of finance and the financial needs of the various supply chain partners in the fruits supply chain in Hyderabad so as to identify points of financial intervention and suggest few measures.

Gaddiannaram market in Hyderabad was selected for the study as it is a major fruit market in the state and has the presence of major wholesalers. The selected market is governed by the Agriculture Market Committee.

Twenty farmers who cultivate mango, papaya and guava were selected from the farmers who were regularly selling their produce in the selected market yard. A sample of 30 retailers, 30 commission agents was selected randomly for collecting the data on selected fruits from Gaddiannaram market.

Trends in wholesale prices and volumes of selected fruits in the selected market

The trends in volumes and prices of the selected fruits in Hyderabad has been worked out. The analysis of trends helps us in knowing the variation of quantities and prices for the years 2014-15 and 2015-16, for the selected fruits mango, papaya and guava.

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Fig .1: Trends in arrivals and prices of Mango in Kothapet market for the 2014-15 and 2015-16

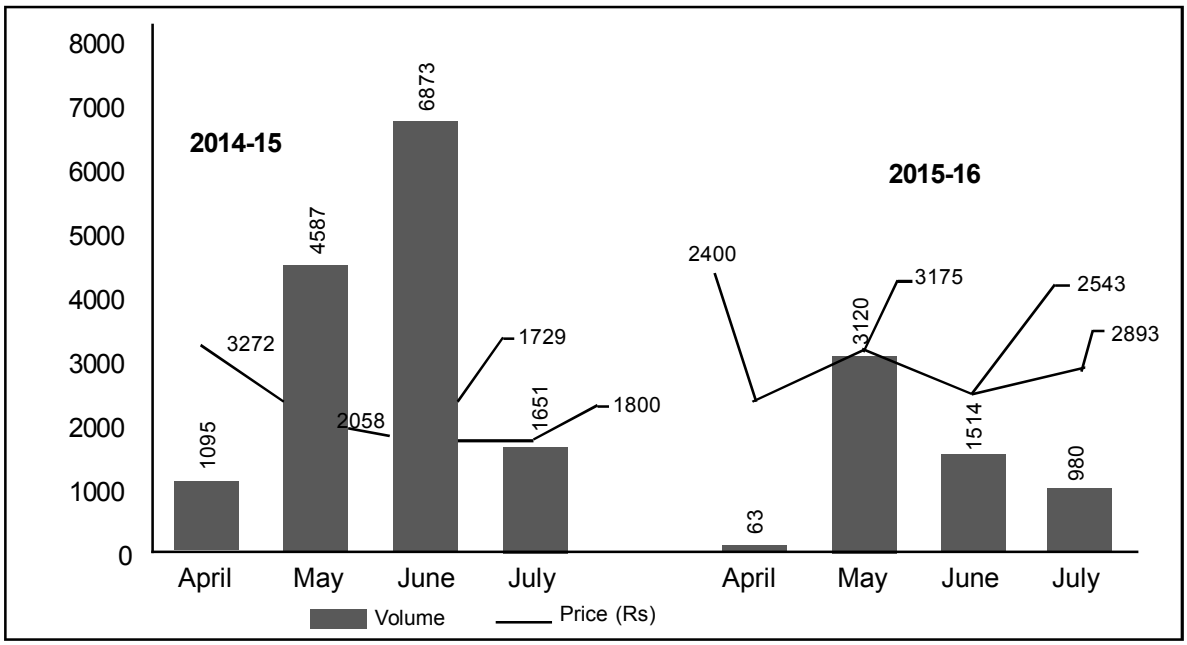
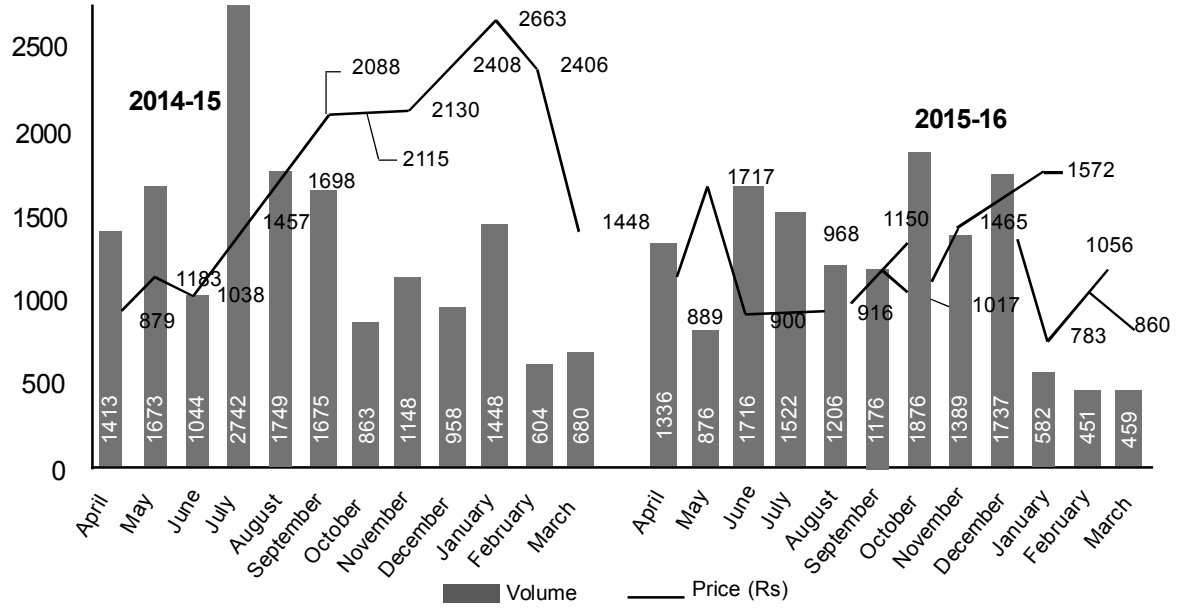


Fig.1 depicts the annual arrivals of mango for the years 2014-15 and 2015- 16. It can be observed that the arrival season of mango was only from April to July. The volumes arrived were highest in the month of June 2014 and the price was highest in the month of April 2014 for the year 2014-15. The volumes arrived were the lowest in the month of April 2015 and the prices were highest during May, 2015 for the year 2015-16.

Fig. 2 : Trends in arrivals and prices of Papaya in Kothapet market for years 2014-15 and 2015-16



It can be noticed that arrivals of papaya are throughout the year in the markets of Hyderabad. Fig. 2 shows the arrivals and prices of papaya for the years 2014-15 and 2015-16. It can be observed that the highest amount of volumes for the year 2014-15 arrived in the month of July 2014 and the highest price was observed in the month of January 2015 for the year 2014-15. The total arrivals were more in the year 2014-15 in comparison to the year 2015-16. In the year 2015-16 the arrivals were more during the June, July and December months and arrivals were very less during February and March months. The prices were high during December and April months.

Fig. 3 : Trends in arrivals and prices of Guava in Kothapet market for years 2014-15 and 2015-16

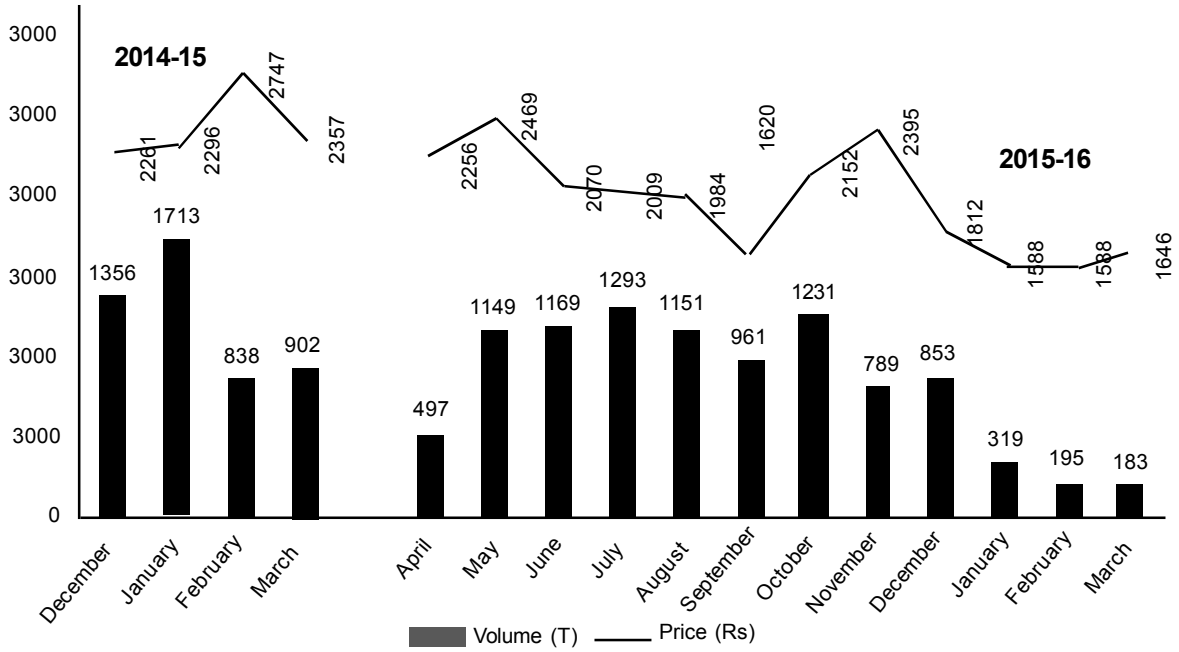
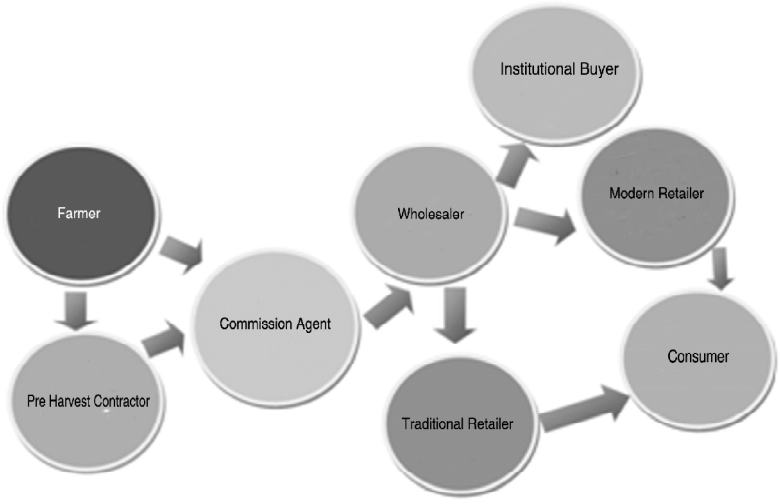


Fig. 3 shows the volumes and prices of guava for the years 2014- 15 and 2015-16. The highest volume of arrivals were seen in the month of January 2015 and the highest price was observed in the month of February 2015 for the year 2014-15. There were no arrivals from April 2014 to November 2014. Hence the total arrivals were very low in the year 2014-15 compared to 2015-16. In the year 2015-16, arrivals were maximum in July. From the analysis of trends in arrivals and prices it can be seen that prices are high when arrivals are high. The findings are in accordance with the study of Kumar *et al* (2009) which mentions that prices lead arrivals

Supply chain of selected fruits

It was observed from the field survey that the supply chains followed were found to be the same for all the selected fruits. The maximum number of partners in the supply chain was six. Figure no. 4 represents the supply chain for selected fruits in Hyderabad market.

Fig . 4 : Supply chain of selected fruits



The supply chains identified in the study area for fruits are as follows:

Channel – I

Farmer → Pre-Harvest Contractor → Commission Agent → Wholesaler → Traditional Retailer → Consumer

Channel – II

Farmer → Pre-Harvest Contractor → Commission Agent → Wholesaler → Modern Retailer → Consumer

Channel – III

Farmer → Pre-harvest Contractor → Commission Agent → Wholesaler → Institutional Buyer

Channel – IV

Farmer → Commission Agent → Wholesaler → Traditional Retailer → Consumer

Channel – V

Farmer → Commission Agent → Wholesaler → Institutional Buyer

Channel – VI

Farmer → Commission Agent → Wholesaler → Modern Retailer → Consumer

Channel – VII

Farmer → Commission Agent → Wholesaler → Consumer

From the above channels we see that the farmers, pre-harvest contractors, wholesalers and the retailers are the major players in the supply chain. In the supply chain of mango and guava, channel-I is the most prominent channel wherein the producers sell their produce to the pre harvest contractors who in turn sell it in the Gaddiannaram market yard through the commission agents. The commission agents charge four per cent commission for the produce sold. The pre-harvest contractors are preferred by the farmers because they will buy the produce at the farm gate and also the farmer becomes free from the risk of price fluctuations.

The next preferred channel was channel-VI, where the farmer directly sold the produce to the wholesalers through the commission agents. The wholesaler then sold it to the modern retailer and thus the produce moved to the consumer from the modern retailer. The grading and sorting will be done by the wholesaler according to the criteria of the modern retailer. The produce that does not meet the quality standard will be returned by the modern retailer to the wholesaler. Channel-VII was also identified as an important channel. Here the farmer sold the produce to the wholesalers through the commission agent and then the wholesalers sell it to the consumers. This channel had the least number of intermediaries among all the channels involved in selling the produce.

In the supply chain of papaya, channel-IV was the most preferred one where the farmers sold their produce to the wholesalers through the commission agents in the market yard and then the produce was bought by the consumers from the traditional retailers. The next preferred channel was channel-VII, while the least preferred channel was channel-I as the farmers did not prefer the post-harvest contractors for selling papaya.

Sources of finance to the supply chain players

All the players of the supply chain need finance for their enterprise to run. There are both formal and informal sources of finance available to the farmers as well as other partners of the supply chain. The formal sources of finance for the farmers are banks and informal sources are mostly money lenders.

The other intermediaries i.e., the commission agents and the retailers have banks- both private and public sector serving as formal sources of finance and friends and family serving as the informal sources of finance.

Table 1. Sources of finance to the farmers

S.No	Nature of Source	Category	Average Amount Borrowed (Rs)	Rate of Interest per Annum	Repayment Period (Years)	Documents Required
1.	Formal	Banks	125000	12	2.2	Bank pass book, Aadhaar card,Land documents & MRO office stamp paper
2.	Informal	Money Lenders	50000	60	3	Nil

The average amount borrowed by the farmers from the formal and informal sources was Rs.125000 and Rs. 50000 respectively. The informal sources were preferred only in case of emergencies as 70 per cent of the farmers were dependent only on banks for their financial needs. The vast difference in the interest rates between the banks and money lenders makes the farmers to look for other formal sources of finance at lower interest rates. Some of the banks that were serving the farmers are Sangameshwara Grameena Vikas Bank, Andhra Bank and State Bank of India.

Table 2 . Sources of finance to the commission agents

S.No	Nature of Source	Category	Average Amount Borrowed (Rs)	Rate of Interest per Annum	Repayment Period (Years)	Documents Required
1.	Formal	Banks	150000	11	2	Bank pass book, Adhaar card, IT returns, Licence, Shop ownership papers
2.	Informal	HandLoans	50000	24	1	Nil

Table No. 2 shows the financial borrowing sources of the commission agents. The average amount borrowed by the commission agents from formal and informal sources is Rs. 150000 and Rs. 50000 respectively. With an average annual turnover of three crores, the commission agents are equally dependent on both the sources for fulfilling financial needs. HDFC, ICICI and Bank of Baroda are few banks which are serving the commission agents in the market.

Table 3. Sources of finance to the retailers

S.No	Nature of Source	Category	Average Amount Borrowed (Rs)	Rate of Interest per Annum	Repayment Period (Years)	Documents Required
1.	Formal	Banks	100000	11	1	Pass Book, Land Documents, Title DD, Adhaar Card MRO Office paper
2.	Informal	Hand Loans	50000	24	1	Nil

The table No 3 shows the sources of finance available to the retailers. The retailers are mostly dependent on informal sources of finance though the rate of interest is high because of the cumbersome paper work involved in the formal sources. Most of them are not educated enough to understand and fulfil the requirements of formal lending.

Financial challenges faced by various supply chain partners

There are varied financial challenges faced by the supply chain partners involved in fruit supply chain, which are listed in the following tables.

Table 4. Financial challenges faced by the fruit retailers

S.No	Particulars	Rank					RBQ	Overall Rank
		I	II	III	IV	V		
1.	Inadequate credit	9	8	6	3	4	69.6	1
2.	Inadequate subsidy	0	8	0	7	15	45.3	4
3.	High interest rate on loan	6	5	8	2	9	57.9	3
4.	Insufficient repayment time	0	0	8	10	12	37.3	5
5.	Difficulty in documentation work	10	6	6	3	5	65.03	2

The various financial challenges faced by the retailers of fruits were listed and ranked according to Rank Based Quotient. It was found that inadequate credit availability was the most basic constraint for retailers. The retailers also faced difficulty in documentation work which restricted them from opting for formal financial sources. High rates of interest on the loan, insufficient subsidy and insufficient repayment time were the other major constraints faced by the retailers.

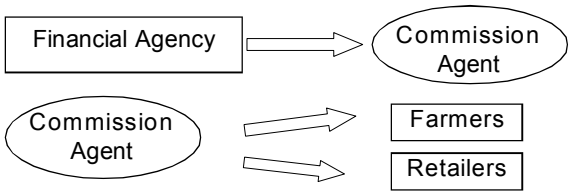
Table 5. Financial challenges faced by the fruit commission agents

S.No	Particulars	Rank					RBQ	Overall Rank
		I	II	III	IV	V		
1.	Inadequate credit	7	5	7	5	6	61.2	2
2.	Inadequate subsidy	2	5	9	8	6	52.5	4
3.	High interest rate on loan	0	0	5	10	15	33.0	5
4.	Insufficient repayment time	5	2	9	10	4	54.9	3
5.	Difficulty in documentation work	10	8	8	4	0	75.9	1

Among the various constraints faced by fruit commission agents difficulty in documentation work was ranked first followed by inadequate credit. Insufficient repayment time was ranked as third constraint. Inadequate subsidy and high rates on interest were the other major constraints for the commission agents. Based on the study, it was felt that, the following two models can be adopted by financial agencies for financial intervention in the supply chain of fruits. The commission agents can be financed directly or they can be taken as guarantor for financing farmers and retailers.

Commission Agent

Model - I



Comission agent can be taken as guarantor for financing retailers and farmers. Retailers who depend upon the daily financers have to pay very high interest rate. The retailers were also of the opinion that they could increase their business by 25 per cent if they get more finance. This factor can be used and monthly finance can be provided instead of daily finance. This would help them in repayment and it would be beneficial to the company in getting the returns. Farmers would also benefit in getting finance as it would bring more transparency in operations because commission agent would also become more cautious as he would be answerable to the company.

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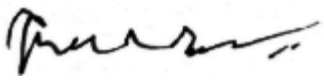

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