

Response of Commercial Rice Germplasm on Agronomic Trait against Seed Contaminants

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Abstract

Rice exhibits a significant share as a part of human food globally as well as in Pakistan. So, it is necessarily important to cope with the ever increasing food requirements through high yield of quality rice by cultivating improved varieties. In conventional conditions where farmers by themselves also prepare there seed from the best lot of standing crop for the upcoming season. In spite of the awareness of the importance of quality seed undesired biological and inert material contamination is experienced. Therefore sample drawn from commercial lots was evaluated against microbial and inert matter contaminants. Test material comprising on five commercial fine and course varieties exhibited wide range of variation in their susceptibility response. The seeds of IR-06 had considerable less inert matter (0.57%), followed by Basmati Super (0.67%), Basmati-2000 (0.85%), KS-282 (2.14%) and KSK-133 (3.28%). Basmati Super possessed highest germination potential of 96% in comparison with Basmati-2000 (92%), IR-06 (86%), KSK-133 (82%) and KS-282 (80%). The 1000 seeds weight of KS-282 was 24 g; while, the KSK-133, IR-06, Basmati Super and Basmati-2000 had 23, 23, 22.5 and 22.5 g; respectively. The comparative study of root and shoot length showed homogenized results, as 4 out of 5 varieties recorded average shoot length up to 15 cm. The remaining one variety (IR-06) was with quite dwarf seedlings. In case of root length again IR-06 was least in its length comparatively; while, the rest having average root length not less then 6.5 cm. KSK-133 attained the maximum average root length of 10 cm. Keen analysis of seed associated mycoflora was resulted in the isolation of *Aspergillus flavus*, *A. niger*, *Penicillium sp.*, *Alternaria spp.*, *Fusarium spp.* and *A. fumigatus*. Moreover study would help the producers for selection of suitable variety in their fields.

Key words: Mycology, Agronomy, Physical Analysis, Seed Pathology.

Introduction

The cultivated rice plant (*Oryza sativa* L.) belongs to the tribe *Oryzaceae* under the sub-family *Pooideae* in the grass family Gramineae (Poaceae) Biosystematics recently divided the genus *Oryza* into several sections and placed *O. sativa* under series Sativa in section Sativae. *O. sativa* is indigenous to Asia. *O. sativa* is a diploid species with 24 chromosomes. Its genomic formula is AA. The rice plant may be characterized as an annual grass, with round, hollow, jointed culms, rather flat, sessile leaf blades, and a terminal panicle. Rice is one of the most important cereals of the world and is consumed by 50% of the total world population (Luo et al; 1998). In Pakistan, it is cultivated on an area of 2581 thousand hectares with total production of 5483 million tons (Anonymous, 2007). This yield is very low as compared to developed countries of the world and this low production is attributed to several biotic and abiotic factors along with the prevailing cultural practices of this region. Among the biotic factors disease is the most important factor which results in crop losses of \$ 5 billion every year (Asghar et al. 2007).

Rice (*Oryza sativa* L.) attains second place among cereal crop, both for acreage and production in the world. It can be an effective weapon for increasing the foreign exchange (Khalid et al. 2003). It accounts for 6.7% in value added in agriculture and 1.6% in GDP of Pakistan. Different varieties of rice are grown in Pakistan for example Super Basmati, Basmati PK-385, IRRI-6, IRRI-9 and KS-282 etc. Pakistan is primarily known for its aromatic rice (Super Basmati, Basmati PK-385, Basmati2000 and Basmati 515). As it is the important staple food so it is very essential to maintain the purity level in its seeds to maintain high quality, disease free and hygienic food production. The importance of seed quality in realizing the full potential of a variety is well known. The three major aspects of seed qualities are **a)** genetic and physical purity, **b)** high germination percentage and vigor, and **c)** free from seed-borne diseases and insects (Eshu and Dadlani, 1989). So testing all these parameters is important for insurance of seed quality. ISTA standards are being followed for the testing of above mentioned parameters throughout the world. *Oryza punctata* "red rice" is considered as the most objectionable weed of rice, at present days internationally. As it has same morphology of seed and same size and it becomes very difficult to remove it from the seed lot, during the processing of paddy rice.

Seed health refers primarily to the presence or absence of disease-causing organisms such as fungi, nematodes, bacteria, viruses and insects associated with seed. Physiological conditions such as trace element deficiency may also affect seed health (ISTA, 1985). Farmers often use seeds that have impurities and contaminants and are infected with pathogens (Fujisaka et al. 1993). Fungal contamination often occurs in the field prior to harvest. Post-harvest contamination can occur if the drying is delayed and during storage of the crop, if moisture is allowed to exceed critical values for mold growth. Delayed harvest in rainy weather frequently leads to grain's sprouting on the panicle, particularly for non-dormant japonica rice have been reported earlier by Reddy and colleagues (2006).

The major storage fungi in rice are *Aspergillus* spp. (Reddy et al. 2004), *Fusarium* sp. (Abbas et al. 1999), *Alternaria* spp. (Gopalakrishnan et al. 2010; Butt et al., 2011 and Utobo et al. 2011) and *Penicillium* sp. (Makun et al. 2007). The harmful effects of such fungal invasion are glume or grain discoloration, loss of viability and quality and toxin contamination. *A. flavus* have been identified as the primary quality deterrent, producing aflatoxin contaminated seeds when in storage explored the incidence of *Aspergillus* spp. (Desai and Ghosh., 2003; Reddy et al. 2005 and Reddy et al. 2008).

Material and Method

The purity related experiment was conducted in the Federal seed certification and Registration Department (FSC&RD) Lahore. The Agronomic traits and mycoflora aspects were studied at the Institute of Agricultural Science, University of The Punjab, Lahore. Three non-basmati and two basmati blood varieties were selected for this work. One kilo gram sample from each lot was taken according to the ISTA standards.

- KSK 282
- KS 133
- IR 06
- BAS SUPER
- BAS 2000

Now these samples were used to draw the working samples in different seeds have different weight, for this purpose the seed sample of about 3000 seeds were obtained from all the 5 seed lots available. According to the ISTA standers the working sample for the rice is 70 gram, (3000 seed approximately) which were obtained by the random distribution of seeds in the mechanical divider available in the FSC&RD. For purity test the working sample was observed under the magnifying lens and following parameters were measured

- The amount of inert mater
- Amount of other crop seeds
- The presence of objectionable weed seed
- And the detection of presence of other variety seeds

Several helpful devices were available in the lab to assert the various separations from a poorly cleaned sample. A set of graduated sieves was used for the separation of different components of seed lot. For this purpose separation blower was also used. The seeds were placed in blower lot by lot. The air flow is directed in upward direction into the tube of specific diameter. The light weight material was blown to the top of the tube, then fall in to a cup near the top of the tube.

Weigh and recorded the weight of working sample or only take the weight of 1000 seeds these seeds will show the vigor of the seed sample.

- After this the working sample was placed on the clean working bench to examine some important attributes
- The variety was identified and confirmed by observing the seed samples under magnifying lens.
- By this procedure inert meter, other crop seeds, infested seeds, weed seeds, objectionable weed seeds are separated from the sample and measured in terms of weight percentage per working sample.

This procedure was repeated for all the 5 seed lots available for testing.

Red rice is the most objectionable weed in paddy so there are very strict standards and zero tolerance for red rice. The presence of red rice was observed more keenly than any other attribute.

Germination Test

This test was performed in Institute of Agricultural Science, University of The Punjab, Lahore as a pot experiment. The sand was fumigated in a covered tab by 10% solution of formalin for 5 to 6 days and pots were also sterilized by spirit. After the fumigation the sand was remained opened for about two days to avoid the residual effect of formalin. The pots containing small hole in the bottom were filled with sand and the gravels at base just to insure the proper drainage of water. The pots were watered and left for one day. After one day ten seeds were sown in each pot. This experiment was done in replication and there were three pots for each type of variety.

Mycoflora Assay

This assay was performed only for the identification of storage fungi associated with seeds. The seeds of all varieties were selected on random bases. First the seeds were surface sterilized to remove any type of surface contamination. The seeds were placed in 2% MEA. The autoclaved MEA media was poured in the plats in sterilized conditions under laminar flow hood. For each variety 3 replicates were made. These plates were incubated in oven at 37 degree Celsius and left untouched. The plates were observed daily. At 3rd day there were multiple colonies visible in each plate. On the complete development of the colonies were further purified and isolated on new 2% MEA media plates. As the growth is now of purified colonies of fungus these pure cultures were send to the First Fungal Culture Bank in Pakistan (FCBP), Institute of Agricultural Sciences, University of The Punjab, Lahore for identification of the colonies. After this the total number of colonies in each plate and the presence of specific fungal colony and their number were calculated to obtain the frequency of occurrence of different fungi.

Data collection

The number of seedling germinated per pot was counted daily. At the final day the pots were watered and the soil were pulled out of pots in a bucket. The plants were uprooted gently to avoid the damage to shoots or roots. Now the shoot length and root length of each plant were measured by using the foot scale. Data was noted in a data sheet and entered in excel book in computer for further analysis. Averages shoot and root length of each plants pot and the average values of these parameters for each variety were calculated. The colonies after purification were identified.

Statistical analysis

All the data were analyzed by one way analysis of variance followed by Duncan's Multiple Range Test to separate the treatment means at $P \leq 0.05$ (Steel et al., 1997).

Results

The results of purity analysis, inert matter, other varieties seeds, objectionable weed seeds (Red Rice), infestation, 1000 seeds weight and moisture tests are described in Table 1. The seed lot is decided to be accepted or rejected after matching these results with standards. All seed lots were passed by FSC&RD as no one had the significant amount of red rice in it.

The germination results show a significant variation in the rate of germination of all the varieties even in the same set of environment. The results are showing maximum % age germination for super basmati rice (96%) and least for KSK 282 (80%). The second highest value was of basmati 2000 (92%) followed by IR- 06 (86%) and KS-133 (82%).

The daily germination pattern shows in the figure 1. The rate of germination for all the considered varieties showed equal behaviour from initial day to 3rd day, after that the number of seedling per pot ranged from 7 to 9 for each variety on an average as showed in the graph but the behaviour of both Basmati variety were quite better as compared to other varieties.

The comparative study of root and shoot length shows that there is a comparatively homogenized results for shoot length as 4 out of 5 varieties showed the average shoot length up to 15 cm the remaining one variety IR-06 showed quite dwarf seedling although all of them were given the same environmental conditions. In case of root length again IR-06 was least in its length comparatively while the rest having the average root length not less than 6.5 cm with KSK-133 attaining the average root length is 10 cm that is maximum.

The mycoflora analysis of seeds for storage and soil fungi variation in results. Among these fungi *Aspergillus* spp. were present in all the samples of different varieties with the most dangerous *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger* in greater % frequency. *Penicillium* sp, *Fusarium* spp. and *Alternaria* spp. were also identified.

Discussion

The purity test results showed maximum level of pure seed in basmati super with a second last lower value of the infested seed presence. The moisture is a parameter which insures the appropriate storage of seed for a long time and in this case all the varieties have the moisture level between 11 to 12 while this is the standard moisture level more moisture creates the chances of fungal and bacterial infection (Jayaraman and Kalyanasundaram., 2009) KSK 282 and IR 06 were advised to have the seed treatment this was because of the reason that during the purity test they showed higher level of infested seeds. Both of these varieties also showed very low root and shoot length this was due to the elevated level of infested seeds. Percentage germination of IR was intermediate but that of KSK 282 also very low.

To indicate as closely as possible the proportion of seed that can sprout and develop in to the plant in field (MacDonald and Copeland., 1989). Therefore the higher rates of germination for basmati rice varieties are the direct indication of better seed vigor and viability. In FSC&RD more emphasis is made in the detection of red rice a famous anxious weed. The mycoflora analysis showed a large amount of *Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus* in most of the samples. These results indicate the poor sanitation conditions of the storage although the moisture level of seed was very ideal, and the seeds were prevented from the excessive detritions. But their association with these seeds shows the threats associated with these fungi release the aflatoxins which can cause serious hazards to the human health. The major storage fungi in rice are *Aspergillus* sp. (Reddy et al., 2004), *Fusarium* ssp. (Abbas et al., 1999) and *Penicillium* ssp. (Makun et al., 2007). The harmful effects of such fungal invasion are glumes or grain discoloration, loss in viability and quality and toxin contamination. Fungal contamination often occurs in the field prior to harvest. Post-harvest contamination can occur if the drying is delayed and during storage of the crop if moisture is allowed to exceed critical values for mold growth. Delayed harvest in rainy weather frequently leads to grain's sprouting on the panicle, particularly rice. The fungi, *A. flavus*, *A. parasiticus*, *A. niger*, and *A. ochraceus*, have been reported earlier by Reddy and colleagues in 2006, of which *A. flavus* have been identified as the primary quality deterrent, producing aflatoxin-contaminated seeds when in storage (Desai et al., 2003; Reddy et al., 2005).

Table 1: Purity Test Results of different varieties of Rice

Variety	Pure seed (%)	Other variety (%)	Inert mater (%)	Red Rice	Infestation (%)	Moisture (%)	1000 seed weight (g)	No. of objectionable weeds/kg
KS-282	97.89	0.07	2.14	0.07	2.82	12	24	1
KSK-133	96.63	0.09	3.28	0.07	4.28	11	23	1
Basmati Super	99.25	.08	0.67	-	.89	12	22.50	-
IR-06	99.34	.09	.57	-	4.14	12	23	-
Basmati-2000	99.06	0.09	0.85	0.05	0.39	12	22.50	-

Varieties	<i>Aspergillus flavus</i>	<i>Fusarium ssp.</i>	<i>Aspergillus niger</i>	<i>Penicillium sp.</i>	<i>Alternari aspp.</i>	<i>Aspergillus fumigatus</i>	Total	% Frequency
KSK-282	2	5	5	1	3	-	16	25.40
KS-133	-	3	2	-	3	2	10	15.87
Basmati Super	4	2	5	-	5	-	16	25.40
IR-06	-	1	2	-	3	3	9	14.29
Basmati-2000	2	2	3	2	1	2	12	19.05
Total	8	13	17	3	15	7	63	
% Frequency	12.70	20.63	26.98	4.76	23.81	11.11		

Table 2: Frequency occurrence of associated fungi with grains of some rice varieties

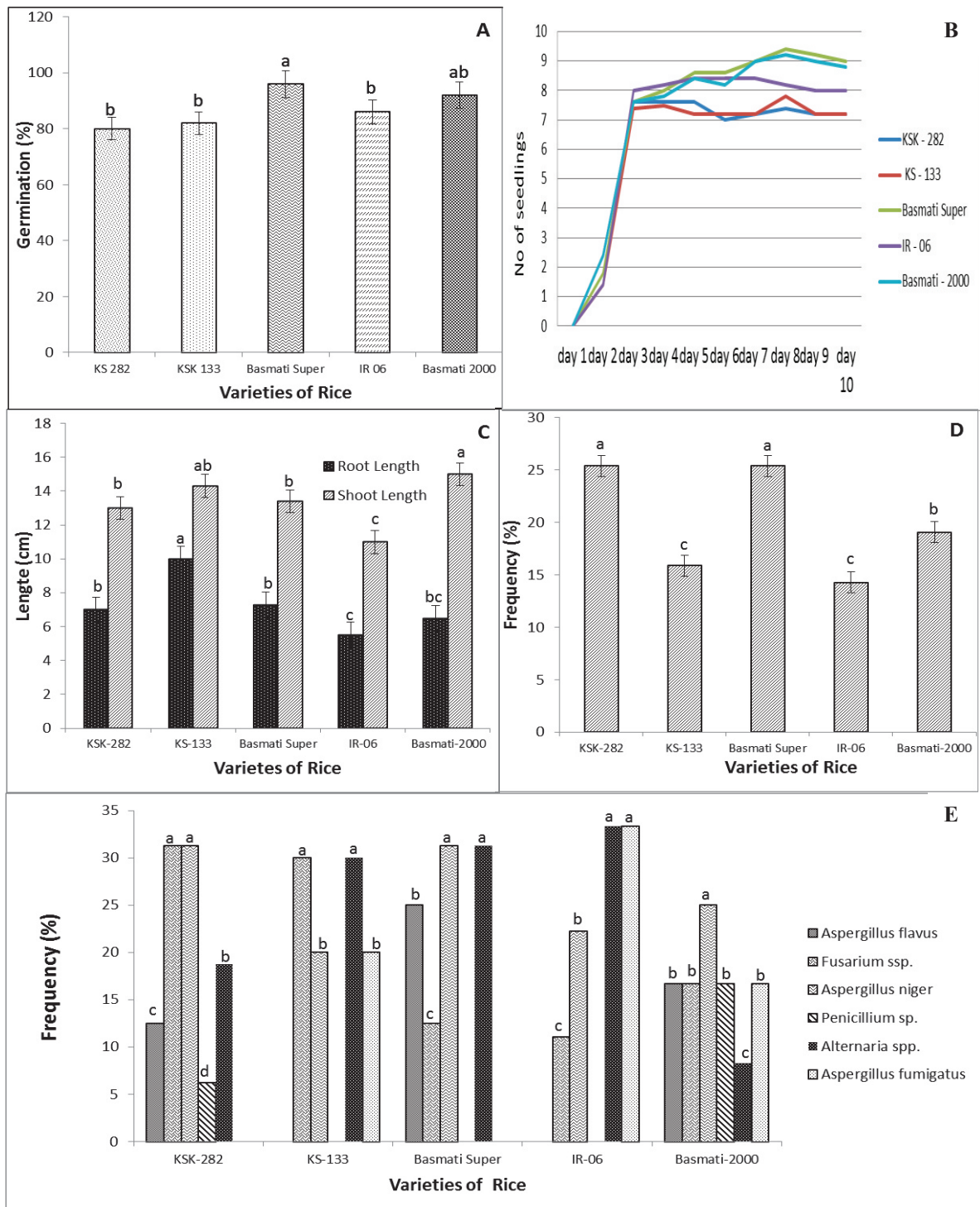


Fig 1: A: Germination percentage. **B:** Germination pattern. **C:** Comparative Root and Shoot length **D:** Percentage mycoflora associated **E:** Occurrence of different seed-borne fungi in five commonly cultivated varieties of rice. Values with different letters show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range test.

Conclusion and Recommendations

Both of the Basmati varieties, Basmati super and Basmati-2000 showed the maximum results in purity and germination test and proved to be the best viable varieties in germination testing. KSK-282, KS-133 and IR-06 showed less impressive results. And due to higher amount of infested IR-6 failed to give the ideal germination rate so among these varieties the lots of basmati varieties were found best and IR-06 as least impressive variety in all of the tested attributes. The presence of aflatoxin producing fungus indicates the poor storage sanitation and the treatment given to the seeds this aspect should be improved.

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