Seed quality evaluation with respect to vigor, viability and fungi associated with commercial rice germplasm

*Maroof Siddique, Noor-ul-Ain Tahir, Sajid Ali, Shumaila Farooq, Hafiza Hamima Elahi Peerzada and Salik Nawaz Khan

Institute of Agricultural Sciences, University of the Punjab, Lahore Pakistan *Corresponding author's email: maroof.iags@gmail.com

Abstract

The present study was conducted to determine the viability and vigor of fourteen rice varieties through tetrazolium test as well as the effect of different growing medium and salt stress on the root and shoot length of these varieties. The presence and location of living tissue within the seeds was evaluated through Tetrazolium (TZ) test. Among the fourteen rice varieties, Basmati 515 showed highest viability, while Basmati 370 showed least viability. Among the different growing substrates, the best performance of root and shoot length was recorded on compost 5.25 and 10.9 cm, respectively. The highest root length against all types of soil achieved by Basmati 515 i.e. 4.5 cm, while Basmati 198 developed least root length of 3.2 cm. The highest shoot length was recorded by PS 2 i.e. 9.65 cm, while KSK 434 showed least shoot length of 6.19 cm. Salinity affects the water uptake by the plants causing reduction in cell turgidity as well as root and shoot elongation. Root and shoot length of rice varieties were evaluated against various electrical conductivity (EC) values from the day of germination and carried up to fourteen days. Seed performance at salt concentrations 1 to 6 ds m⁻¹ were made with reference to higher salt concentration of Rice belt of Punjab province. The highest root length at all salt concentrations was achieved by Basmati Pak i.e. 4.17, while KSK 133 developed least root length 3.03 cm. Variety Basmati Pak developed highest shoot length 6.33 cm, while KSK 133 developed least shoot length 4.88 cm. The study revealed the effectiveness tetrazolium test with respect to time saving and reliability reflecting field performance.

Keywords: Germplasm, Salt Stress, Seedling Emergence, Tetrazolium Test, Viability.

Introduction

Soil physical and chemical properties, land preparation seedling age, seed quality, plant density, vigor, nutrient and pest management are among the important factors that contribute towards good crop yield. However, vigor and good quality of the seeds are the most desirable factors for farmers to get optimum stand establishment but certain environmental and storage conditions can lead to loss of quality (Wang et al., 2010; Kapoor et al., 2011; Mahender et al., 2015). Therefore, root and shoot development aspect of seed quality focus of certain activities that efficiently evaluate the potential of seed lot for successful production. Therefore, the cumulative assessment of seed quality is made against various types of stress like soil type, water, salt concentration etc. according to the growing conditions of crop (Daniela et al., 2017). Vigor tests considered as key tools as well as crude assessment of potential of seed lot quality. Common seed vigor tests are seedling growth (seedling vigor classification and seedling growth rate), stress tests (accelerated aging, cold and cool) and biochemical tests (tetrazolium and electrical conductivity) (ISTA, 2013; AOSA, 2014). However, there is no single vigor test that is universally accepted for all plant species *i.e.* Blotter paper, paper towel and sand bed tests. These methods are generally providing guideline to grower for selection of variety. Proper

interpretation of these tests also provides information that helps in selection of better seed lots, determination of the required quantity of seeds and its distribution during sowing and estimation of probable commercial success under various field condition (Khan *et al.*, 2010; Martins *et al.*, 2014). Therefore, following investigation was conducted with objective to evaluate vigor and viability of rice seed through different vigor test.

Material and Methods

Certified commercial germplasm used in the investigation comprised of fine Basmati germplasm *viz.* Basmati super, Basmati 515, Basmati 370, Basmati 385, Basmati 2000, Basmati Pak, Kisan Basmati and Basmati 198 and fine Non-Basmati comprises PS 2 and PK 386 and coarse germplasm *viz.* KSK 434, IR 6, KSK 133 and KSK 282, and these were procured from Rice Research Institute Kala Shah Kaku (RRI, KSK), Lahore, Pakistan.

Tetrazolium test

The efficacy of tetrazolium test (TZ) was evaluated on both Fine and coarse varieties, seeds of each variety were soaked overnight in distilled water and dried on pre-sterilized blotter paper. The seeds were cut one fourth through the embryo and endosperm. A total of 25 seeds were placed in Petri dish and 30 mL tetrazolium solution (1%) was poured in Petri dish. Then the plates were incubated at 30 °C for 2 hours. Change of embryo color on treatment with tetrazolium was made in three replicated trails against fine and course varieties of rice and observations were recorded at 30 min of intervals.

Growing media

To evaluate the suitable growing media for the vigor of rice germplasm a set different growing media comprising on sterilized soil, fine river sand, field soil, silt, compost, compost + field soil was $2/3^{rd}$ filled in 8×12 cm² pots. The test pot was kept three replicates and placed in natural field environment. The data for germination and root shoot development was recorded after 15 days.

Salt concentrations (SC)

Due to the saline nature of soil of rice belt of Punjab, the test material was also checked against Na salt concentrations of 1 ds m⁻¹, 2 ds m⁻¹, 3 ds m⁻¹, 4 ds m⁻¹, 5 ds m⁻¹, 6 ds m⁻¹, respectively, as field performance indicator performance indicator (Acosta-Motos *et al.*, 2015) For the control treatment, distilled water was applied. All treatments were replicated thrice and kept under controlled conditions of 27 °C in programmable growth chamber, model no. WCG-450 (DAIHAN Scientific co., Ltd, Korea). Data of shoot and root growth were recorded after 15 days.

Isolation of seed borne mycoflora

The germplasm of rice was surface sterilized with 2% sodium hypochlorite for 2 minutes and rinsed two to three times with double distilled water and dried on sterilized blotter paper. Twenty-five seeds were placed on potato dextrose agar (PDA) media plates and incubated at 25 ± 2 °C for 3 days. The fungal distribution and percentage frequency of mycoflora were recorded using the formulae given by Gaddeyya *et al.* (2012).

Frequency (%) = $\frac{\text{No. of CFUs of a species from a variety}}{\text{Totanl No. of CFUs of all species from a variety}} \times 100$

For the microscopic studies, front and back color of colony, conidia size, shape, formation and mycelial morphological features were observed and identified the colonies by specific keys (Goettel and Inglis, 1997; Barnett and Hunter, 1999; Domsch *et al.*, 2007; Humber, 2012).

Data analysis

The collected data were statistically analyzed through analysis of variance (ANOVA) using Statistix 8.1 software. The varietal and treatments means were compared with each other through LSD at 0.05 probability level.

Results and Discussion

Tetrazolium test

Viability of Basmati 515 presented statistically significant response to the interaction of all fine varieties Basmati 515 showed the maximum viability (96%), which was statistically higher than Basmati 370 that showed minimum viability (74.67%) in Fig. 1. Viability of the coarse germplasm showed statistically significant response to the interaction of varieties. PS 2 exhibited the maximum viability 93.33% which was statistically higher, and PK 386 showed minimum viability 77.33% which was statistically lower than other than other coarse varieties respectively in Fig. 2. Fine rice exhibited quick reaction to changing the embryo color from white to red. This change appeared within 30 min and after 60 to 90 min they turned into pink to red color, coarse germplasm achieved pink to red color within 60 to 120 min respectively (Fig. 3).

Growing media

The performance of seed is directly depending on the growing medium, which gives the opportunity to seeds to show their best performance under natural conditions. Among all fine varieties the Basmati 515 shown in Table 1 the statistically significant growth of shoot 14.67 cm in the Compost medium and Basmati 198 had the lowest shoot growth 2.37 cm in field soil. Among the coarse varieties the PS 2 in compost produced maximum shoot growth 17.93 cm which shown in Table 2 the significant than other varieties and KSK 434 produced minimum shoot growth 1.23 cm in field soil.

The performance of seed is directly depending on the growing medium, which gives the opportunity to seeds to show their best performance under natural conditions. Among all fine varieties with the maximum root development of 6.13 cm of Basmati 515 in compost medium which are statistically significant then other varieties (Table 3). The B.198 had least root growth 1.6 cm in silt medium. Among the coarse varieties the PS 2 in compost produced maximum shoot growth 5.67 cm which shown in Table 4 which are significant than other coarse varieties and KSK 434 and IR-6 produced minimum root growth 1.67 cm and 1.61 cm in silt medium.

Salt concentrations

Due to the sodic soil nature of Pakistan the vigor of germplasm was evaluated under the different concentrations of salt stress. The performance of the shoot shown in Table 5 the Basmati Pak had statistically significant growth of shoot 8 cm in 0 ds m⁻¹ and the Basmati 515 had minimum shoot growth in 6 ds m⁻¹. Among the coarse varieties the PS 2 shown in table 6 the maximum shoot length 8.27 cm in 0 ds m⁻¹ which had significant then others and KSK. 133 had minimum shoot growth 2.73 cm under the 6 ds m⁻¹

concentration.

Root length of fine and coarse germplasm is shown in Table 5 and Table 6, respectively. In the fine germplasm the B. 370 had maximum and minimum root growth in 2 ds m⁻¹ and 6 ds m⁻¹, respectively. In coarse germplasm the root length of PS 2 had 5.00 cm which are statistically significant in 0 ds m⁻¹ and the KSK. 133 had minimum root length 2.13 cm in 6 ds m⁻¹ salt concentration.

Percentage contribution and frequency of seed borne mycoflora

A total of eight fungal species were isolated, *Fusarium oxysporum*, *Fuasrium* sp., *Alternaria alternata*, *Penicillium* sp., *Aspergillus flavus*, *Aspergillus* sp. and *Aspergillus niger*. It was observed that among isolated fungal species the *Aspergillus niger* exhibited highest percentage contribution (30.45%) while *Fusarium* sp. had the minimum of 4.10% (Table 9). The highest frequency of fungal specie association was recorded for *Aspergillus flavus* (39.68%) on basmati super whereas percentage frequency of *Fusarium* sp., *Penicillium* sp. and *Aspergillus terreus* were 1.59%, 1.59% and 1.33% were recorded from the KSK. 282, Basmati 198, and KSK 133, respectively (Table 10).

Seed vigor as a quality attribute reflects physiological seed characteristic which determine reliability of seed under their respective growing conditions (Khajeh-Hosseini et al., 2003). There are a number of routine and high-tech lab techniques to evaluate seed quality. Therefore, the stakeholder of seed trades always in struggling in identifying economical, efficient, easy operating and reliable performance methods for seed quality assessment Seedling establishment and demands development of certain methods to identify differences among seed lots according to their physiological potential. Under optimal conditions vigor testing is considered the key factor to get information and solution of the problems during the production process (Bertolin et al., 2011; Pervez et al., 2009).

Two criteria have set by ISTA for vigor tests to be acceptable the first set rule for vigor test is that it can be repeated and has enough uniformity in terms of obtained results. The second rule is that the results should correlate with the results of seedling growth in the field. TZ test determines the percentage of viable seeds by the activity of the dehydrogenase enzyme system regardless of the dormancy level of seeds (Elias *et al.*, 2012).

There was a wide range of variations in germination and seedling sensitivity salt stress levels of lower and higher levels. The varietal difference in rice germplasm against different salt concentrations appeared on quality attributes of emerging seedlings have been reported (Thu et al., 2020). In present investigation of chemical stress, the varietal germplasm was treated with Na salt concentrations of 1 ds m⁻¹, 2 ds m⁻¹, 4 ds m⁻¹ and 6 ds m⁻¹, respectively. Vigor and viability data were recorded after 15 days. Statistically significant response between seedling growth was observed. Among fine varieties Basmati Pak and Basmati 515 showed maximum 6.33 and minimum 4.37 cm shoot length of when grown in 0 ds m⁻¹ or control. In case of coarse varieties interaction of PS 2 when grown in 0 ds m⁻¹ or control produces maximum shoot growth 6.47 cm which was statistically higher whereas KSK 133 produced minimum 4.88 cm shoot length. Among the fine varieties maximum root growth 4.17 cm was recorded for Basmati Pak when grown in 2 ds m⁻¹ and in case of coarse rice maximum 4.03 cm root length was recorded for PS 2 at 1 ds m⁻¹ while minimum root growth 3.03 was observed for KSK 133 at 6 ds m⁻¹ of Na salt. Results of this research indicated that vigor testing is significant tool to predict the performance of seed lot under different environmental condition and can be considered as most desirable characteristic for better yield of rice crop therefore more new more advance methods should be incorporated in rice vigor testing. Fungi associated with crop Seed plays significant role determining seed quality and crop loss therefore in this study data of seed borne fungi from rice germplasm was recorded and different seed borne fungi were isolated from tested rice germplasm. These fungal species have been reported earlier to be responsible for several nursery and field diseases in rice crop (Ibaim et al., 2006). The findings of this investigation provide evidence that mycoflora irrespective of variety and environmental condition caused serious damage to seed quality and transmits diseases that are difficult to control. Moreover, the long term surviving nature of seed borne pathogen also reduces seed viability and damage genetic integrity so all these factor make these fungal species a potential threat to crop production when germplasm is used for plantation and propagation purpose after long period storage (Duan et al., 2007). Therefore, practice of seed health testing is important to reduce diseases and crop loss.

 Table 1: Shoot length (cm) of fine rice germplasm under different growing media.

Variety/Treatment	Silt	Sand	Compost	Field Soil	Field soil +	Variety
					Compost	mean
B. 2000	4.37 l-p ±	8.73 e-I ±	10.17 c-e ±	3.23 n-p ±	6.7 i-k ±	6.64 CD
	0.15	1.62	2.03	1.05	1.51	
B. 385	3.53 m-p ±	9.17 d-g ±	11.27 bc \pm	3.7 m-p ±	7.48 f-j ±	7.03 BC
	0.32	0.15	1.11	0.72	0.36	

B. Super	5.13 k-n ±	8.57 e-I ±	12.77 ab ±	4.2 l-p ± 0.26	8.48 e-I ±	7.83 B
B. 515	$5.2 \text{ k-n} \pm 0.26$	$10.83 \text{ b-d} \pm 0.59$	$14.67 \text{ a} \pm 3.17$	4.87 k-o ± 0.25	9.77 c-f ±	9.07 A
Basmati Pak	4.33 l-p ± 0.32	$9.77 \text{ c-f} \pm 0.76$	$12.53 \text{ b} \pm 0.87$	$3.03 \text{ op } \pm 0.50$	7.78 f-j ± 0.29	7.49 BC
B. 370	4.03 m-p ± 0.96	8.9 d-h ± 0.36	9.63 c-f ± 3.67	3.9 m-p ± 1.59	6.77 i-k ± 2.45	6.65 CD
Kisan Basmati	3.6 m-p ± 0.35	8.03 f-j ± 0.85	8.5 e-I ± 3.69	3.8 m-p ± 0.68	6.15 j-l ± 2.01	6.02 DE
B. 198	3.17 n-p ± 0.86	6.93 h-k ± 1.01	8.43 e-I ± 1.11	2.37 p ± 0.38	5.4 k-m ± 0.72	5.26 E
Growth medium mean	4.17 D	8.87 B	10.996 A	3.64 D	7.32 C	

Table 2: Shoot length (cm) of coarse rice germplasm under different growing media.

Variety/Treatment	Silt	Sand	Compost	Field Soil	Field soil +	Variety
					Compost	mean
PS 2	4.27 j-l ±	9.63 b-e ±	17.93 a ±	$4.97 \text{ h-l} \pm$	11.45 b \pm	9.65 A
	0.90	0.74	0.79	0.72	0.18	
PK. 386	3.97 kl ±	8.23 c-g ±	10.67 b-d \pm	3.4 k-m \pm	$7.03 \text{ f-h} \pm$	6.66 B
	0.15	1.45	2.73	0.79	1.44	
KSK. 434		$8.4 \text{ c-f} \pm$	11.73 b ±	$1.23 \mathrm{m} \pm$	6.48 f-j ±	6.19 B
	$3.1 \text{ lm} \pm 0.98$	0.72	2.71	0.15	1.41	
IR. 6		7.43 e-h \pm	$8.17 \text{ d-g} \pm$	3.47 k-m \pm	$5.82 \text{ g-k} \pm$	5.597 B
	$3.1 \text{ lm} \pm 0.98$	1.16	2.38	0.85	1.12	
KSK. 133	$3.27 \text{ lm} \pm$	7.53 e-g ±		$2.6 \text{ lm} \pm$		6.24 B
	0.50	1.86	$11 b \pm 1.47$	0.92	$6.8 \text{ f-I} \pm 0.28$	
KSK. 282	4.33 i-l ±	7.63 e-g ±	10.7 bc \pm	$2.7 \text{ lm} \pm$		6.41 B
	0.99	2.30	1.75	0.36	$6.7 \text{ f} \cdot \text{j} \pm 0.95$	
Growth medium	3.672 C	8.14 B	11.7 A	3.39 C	7.34 B	
mean						

Table 3: Root length (cm) of fine rice germplasm under different growing media.

Variety/Treatment	Silt	Sand	Compost	Field Soil	Field soil +	Variety
			F		Compost	mean
B. 2000	2.2 m-o ±	$4 \text{ c-j} \pm 0.26$	5.1 a-d \pm	$1.87 \text{ no} \pm$	3.48 h-m ±	3.33 CD
	0.26		0.95	0.50	0.46	
B. 385	2.23 m-o ±	3.43 h-m \pm	5.03 a-e \pm	2.47 l-o \pm	$3.75 \text{ d-l} \pm$	3.38 CD
	0.15	0.35	1.80	0.83	1.30	
B. Super	2.6 k-o \pm	$3.4 \text{ h-m} \pm$	5.43 ab \pm	2.57 l-o \pm	4 c-j ± 0.82	3.6 B-D
	0.26	0.26	1.00	0.65		
B. 515	3.17 i-n ±	$4.93 \text{ a-g} \pm$	6.13 a ±	$3.6 \text{ f-l} \pm$	4.87 a-g \pm	4.54 A
	0.21	0.61	1.68	0.82	1.19	
Basmati Pak	3.33 h-m \pm	$4.33 \text{ b-I} \pm$	5.37 ab \pm	2.5 l-o \pm	$3.93 \text{ c-k} \pm$	3.89 BC
	0.49	0.81	1.07	0.79	0.90	
B. 370	3.17 i-n ±	$3.53 \text{ g-m} \pm$	4.87 a-g \pm	2.53 l-o ±	$3.7 \text{ e-l} \pm 1.07$	3.56 B-D
	0.38	0.25	1.57	0.57		
Kisan Basmati	2.47 l-o ±	5.23 a-c \pm	5.43 ab \pm	2.87 j-o ±	4.15 b-j ±	4.03 AB
	0.36	0.46	1.40	0.92	1.16	
B. 198	$1.6 \text{ o} \pm 0.10$	$4 \text{ c-j} \pm 0.60$	$4.6 \text{ b-h} \pm$	2.4 l-o \pm	$3.5 \text{ h-m} \pm$	3.22 D
			0.98	0.70	0.30	
Growth medium	2.595 C	4.11 B	5.25 A	2.6 C	3.92 B	
mean						

Variety/Treatment	Silt	Sand	Compost	Field Soil	Field soil + Compost	Variety mean
PS 2	3.5 c-h ±	$3.5 \text{ c-h} \pm$	5.67 a ±	3.3 c-j ±	4.48 a-c ±	4.09 A
	0.32	0.85	1.60	0.95	0.33	
PK. 386	1.97 kl ±	3.9 c-e ±	$4.27 \text{ b-d} \pm$	2.37 g-l ±	3.32 c-j ±	3.16 BC
	0.59	0.53	0.85	0.67	0.75	
KSK. 434	$1.67l\pm0.25$	3.43 c-I ±	5.2 ab ±	2.1 j-l ±	$3.65 \text{ c-f} \pm$	3.21 BC
		0.40	1.05	0.75	0.83	
IR. 6	1.61 ± 0.36	3.43 c-I ±	3.53 c-h \pm	$2.5 \text{ f-l} \pm$	$3.02 \text{ d-k} \pm$	2.82 C
		0.40	1.30	1.06	0.74	
KSK. 133	$2.1 \text{ j-l} \pm 0.26$	$2.7 \text{ e-l} \pm$	5.3 ab ±	$2.2 \text{ i-l} \pm$	$3.75 \text{ c-f} \pm$	3.21 BC
		0.44	1.20	0.56	0.75	
KSK. 282	2.07 j-l ±	$3.63 \text{ c-g} \pm$	5.2 ab ±	2.33 h-l \pm	3.77 c-f ±	3.4 B
	0.42	0.38	0.95	0.86	0.35	
Growth Medium	2.4 C	3.82 B	5.08 A	2.54 C	2.4 B	
Mean						

Table 4: Root length (cm) of coarse rice germplasm under different growing media.

Table 5: Shoot length (cm) of fine rice germplasm under salt concentrations.

Variety/Treatment	0 ds m ⁻¹	1 ds m ⁻¹	2 ds m ⁻¹	4 ds m ⁻¹	6 ds m ⁻¹	Variety
variety/freatment	0 us m	1 us m	2 us m	4 us m	0 us m	mean
B. 2000	$7.1~b\pm0.30$	6.7 b-f ±	$6.33 \text{ b-h} \pm$	$5 \text{ m-p} \pm$	3.63 rs ±	5.75 B
		1.04	0.40	0.40	1.00	
B. 385	$6.73 \text{ b-f} \pm$	$6.03 \text{ f-k} \pm$	6.37 b-g ±	6.2 d-j ±	$4.4 \text{ p-r} \pm$	5.95 B
	0.35	1.29	0.35	0.62	0.40	
B. Super	6.53 b-g ±	5.53 i-n \pm	5.33 k-o \pm	$6.03 \text{ f-k} \pm$	5.33 k-o ±	5.75 B
-	0.51	0.06	0.25	0.25	0.25	
B. 515	5.27 k-o \pm	4.9 m-p ±	4.77 n-q ±	$4 qr \pm 0.36$	$2.9 \text{ s} \pm 0.66$	4.37 D
	0.15	1.14	0.80			
Basmati Pak	$8.00 a \pm 0.56$	$6.93 \text{ b-d} \pm$	$6.33 \text{ b-h} \pm$	4.93 m-p ±	5.43 j-o ±	6.33 A
		0.74	0.25	0.38	0.21	
B. 370	6.13 e-j ±	$5.87 \text{ g-l} \pm$	5.57 h-m \pm	4.73 o-q ±	4.73 o-q ±	5.41 C
	0.31	0.31	0.50	0.25	0.51	
Kisan Basmati	7.07 bc \pm	$6.3 \text{ c-I} \pm$	5.5 j-o ±	4.37 p-r ±	5.1 l-p ±	5.67 BC
	0.15	0.25	0.79	0.75	0.55	
B. 198	6.87 b-e \pm	5.3 k-o \pm	$5.3 \text{ k-o} \pm$	$6.47 \text{ b-g} \pm$	$4.83 \text{ m-p} \pm$	5.75 B
	0.15	0.36	0.56	0.32	0.46	
SC means	6.71 A	5.95 B	5.69 B	5.21 C	4.55 D	

 Table 6: Shoot length (cm) of coarse rice germplasm under salt concentrations.

Variety/Treatment	0 ds m ⁻¹	1 ds m ⁻¹	2 ds m ⁻¹	4 ds m ⁻¹	6 ds m ⁻¹	Variety mean
PS 2		6.67 b-e ±	$6.5 \text{ b-f} \pm$	5.6 i-k ±	5.33 j-l ±	6.47 A
	$8.27 a \pm 0.15$	0.36	0.56	0.32	0.46	
PK. 386	$6.83 \text{ bc} \pm$	$6.2 \text{ d-I} \pm$	$6.4 \text{ c-g} \pm$	$5.17 \text{ k-m} \pm$	5.97 f-I \pm	6.11 B
	0.35	0.26	0.36	0.15	0.15	
KSK. 434	6.8 b-d \pm	$5.17 \text{ k-m} \pm$	$6.03 \text{ f-I} \pm$	4.9 l-n \pm	$4.6 \text{ mn} \pm$	5.5 C
	0.20	0.15	0.42	0.66	0.53	
IR. 6	$6.33 \text{ c-h} \pm$	$6.33 \text{ c-h} \pm$	5.8 g-j ±	$5.73 \text{ h-k} \pm$	5.23 j-k ±	5.89 B
	0.15	0.15	0.30	0.21	0.40	
KSK. 133	$6.57 \text{ b-f} \pm$	$6.33 \text{ c-h} \pm$	$4.47n \pm$	4.3 n ±		4.88 D
	0.38	0.15	0.15	0.30	$2.73 \text{ o} \pm 0.51$	
KSK. 282	$6.77 \text{ b-d} \pm$	6.13 e-I ±	$7.07 b \pm$	$6.1 \text{ e-I} \pm$	$4.6 \text{ mn} \pm$	6.13 B
	0.32	0.31	0.42	0.10	0.10	
SC means	6.93 A	6.13 B	6.04 B	5.3 C	4.74 D	

Variety/Treatment	0 ds m ⁻¹	1 ds m ⁻¹	2 ds m ⁻¹	4 ds m ⁻¹	6 ds m ⁻¹	Variety mean
B. 2000	3.03 jk ±	4.57 a-d \pm	$4.1 \text{ b-g} \pm$	4.53 a-d \pm	$2.87 \text{ kl} \pm$	3.82 AB
	0.21	1.27	0.26	0.21	0.50	
B. 385	3.23 i-k ±	4.57 a-d \pm	$4.1 \text{ b-g} \pm$	3.97 c-I ±	$3.37 \text{ g-k} \pm$	3.85 AB
	0.21	1.27	0.26	0.15	0.15	
B. Super	$3.37 \text{ g-k} \pm$	$3.63 \text{ e-k} \pm$	$3.47 \text{ f-k} \pm$	$4.17 \text{ b-g} \pm$	$4.63 \text{ a-d} \pm$	3.85 AB
	0.06	1.10	0.06	0.15	0.32	
B. 515		3.5 f-k ±	$4.37 \text{ b-e} \pm$	3.97 c-I ±		3.95 A
	$4.2 \text{ b-f} \pm 0.20$	0.10	0.21	0.15	$3.7 \text{ e-j} \pm 0.95$	
Basmati Pak		4.57 a-d \pm	$3.57 \text{ e-k} \pm$	$3.47 \text{ f-k} \pm$	4.33 b-e ±	4.17 A
	$4.9 \text{ ab} \pm 0.26$	1.19	0.15	0.21	0.25	
B. 370	3.03 jk ±	$3.43 \text{ f-k} \pm$	5.23 a \pm	$4.03 \text{ c-I} \pm$		3.57 B
	0.06	0.15	0.35	0.25	2.131 ± 0.31	
Kisan Basmati	$3.67 \text{ e-k} \pm$	3.83 d-j ±	$4.17 \text{ b-g} \pm$	$4.07 \text{ c-h} \pm$	$3.4 \text{ f-k} \pm$	3.83 AB
	0.15	0.36	0.57	0.06	0.25	
B. 198	4.77 a-c \pm	$3.87 \text{ c-I} \pm$	$4.17 \text{ b-g} \pm$	3.97 c-I ±	$3.27 \text{ h-k} \pm$	4.00 A
	0.15	0.50	0.06	0.15	0.35	
SC means	3.76 B	3.995 AB	4.15 A	4.02 AB	3.46 C	

Table 7: Root length (cm) of fine rice germplasm under salt concentrations.

Table 8: Root length (cm) of coarse rice germplasm under salt concentrations.

Varietv/Treatment	0 ds m ⁻¹	1 ds m ⁻¹	2 ds m ⁻¹	4 ds m ⁻¹	6 ds m ⁻¹	Variety
						mean
PS 2	$4.37 \text{ bc} \pm$	$5.00 a \pm$	$3.77 \text{ d-h} \pm$	3.43 f-k ±	3.57 f-j ±	4.03 A
	0.25	1.11	0.15	0.31	0.20	
PK. 386	3.33 g-k ±	$4.37 \text{ bc} \pm$	$3.37 \text{ g-k} \pm$	3.13 j-l ±	3.9 c-g ±	3.62 B
	0.15	0.40	0.35	0.12	0.10	
KSK. 434	$3.5 \text{ f-k} \pm 0.10$	$3.73 \text{ e-I} \pm$	$3.77 \text{ d-h} \pm$	$3 j-1 \pm 0.20$	$3.27 \text{ h-k} \pm$	3.45 B
		0.31	0.32		0.42	
IR. 6	$3.77 \text{ d-h} \pm$	$3.53 \text{ f-k} \pm$	$4.17 \text{ b-e} \pm$	$4.33 \text{ b-d} \pm$	4.3 b-e ±	4.02 A
	0.15	0.21	0.31	0.15	0.20	
KSK. 133	3.33 g-k ±	3.03 j-l ±	3.17 i-l ±	$2.97 \text{ kl} \pm$	2.631 ± 0.72	3.03 C
	0.15	1.00	0.60	0.21		
KSK. 282	3.17 i-l ±	$3.53 \text{ f-k} \pm$	4.5 ab ±	$3.97 \text{ b-f} \pm$	3.37 g-k ±	3.71 B
	0.21	0.31	0.30	0.21	0.21	
SC means	3.58 BC	3.87 A	3.79 AB	3.47 C	3.51 C	

 Table 9: Percentage contribution of mycoflora associated with germplasm of rice.

Varieties	No of colonies	Fo	F sp	Aa	P sp	Af	A sp	An	At
B. 2000	15.00	1.67 ±	$0.33 \pm$	$0.33 \pm$	$1.67 \pm$	$5.00 \pm$	$0.67 \pm$	$5.00 \pm$	0.33 ±
		1.03	0.52	0.52	0.52	1.79	0.52	1.55	0.52
B. 385	19.33	$3.00 \pm$	$1.00 \pm$	$2.00 \pm$	$0.00 \pm$	$5.33 \pm$	$1.33 \pm$	$5.67 \pm$	$1.00 \pm$
		0.00	0.89	0.89	0.00	1.37	1.03	1.03	0.89
B. Super	11.33	$1.33 \pm$	$0.67 \pm$	$1.00 \pm$	$0.33 \pm$	$5.00 \pm$	$0.33 \pm$	$2.67 \pm$	$0.00 \pm$
		1.03	0.52	0.00	0.52	1.55	0.52	2.07	0.00
B. 515	16.00	$1.00 \pm$	$1.00 \pm$	$0.33 \pm$	$0.67 \pm$	$6.00 \pm$	$2.33 \pm$	$4.33 \pm$	$0.33 \pm$
		0.00	0.89	0.52	0.52	0.89	0.52	1.86	0.52
B. 6129	15.00	$2.00 \pm$	$0.67 \pm$	$1.6 \pm$	$1.00 \pm$	$4.33 \pm$	$0.67 \pm$	$4.00 \pm$	$0.67 \pm$
		1.55	0.52	1.37	0.00	1.03	0.52	1.79	0.52
B. 370	15.67	$0.33 \pm$	$0.33 \pm$	$0.67 \pm$	$0.67 \pm$	$5.33 \pm$	$2.33 \pm$	$5.33 \pm$	$0.67 \pm$
		0.52	0.52	0.52	0.52	1.37	0.52	1.03	0.52
Kisan	15.00	$0.67 \pm$	$0.00 \pm$	$0.00 \pm$	$0.33 \pm$	$4.67 \pm$	$2.67 \pm$	$6.00 \pm$	$0.67 \pm$
Basmati		0.52	0.00	0.00	0.52	1.37	1.86	0.89	1.03
B. 198	16.33	$2.00 \pm$	$0.67 \pm$	$0.67 \pm$	$0.33 \pm$	$4.67 \pm$	$1.00 \pm$	$5.33 \pm$	$1.67 \pm$

	$F_{0} = Fusarium oxysporum$				F sp = <i>Fusarium</i> sp				
Contri- bution (%)	10.83	4.	10	4.83	5.42	32.21	8.49	30.45	3.66
Total	227.67	24.67	9.33	11.00	12.33	73.33	19.33	69.33	8.33
282		0.52	0.52	1.37	0.00	1.03	0.52	0.52	0.52
KSK.	19.00	$2.33 \pm$	$0.33 \pm$	$1.33 \pm$	$1.00 \pm$	$5.67 \pm$	$2.33 \pm$	$5.33 \pm$	$0.67 \pm$
133		1.37	0.89	0.52	0.52	0.52	0.89	0.52	0.52
KSK.	20.33	$2.33 \pm$	$1.00 \pm$	$0.33 \pm$	$2.33 \pm$	$6.33 \pm$	$1.00 \pm$	$6.67 \pm$	$0.33 \pm$
		0.89	0.52	0.89	0.00	1.55	0.52	1.03	0.89
IR. 6	15.67	$2.00 \pm$	$0.67 \pm$	$1.00 \pm$	$1.00 \pm$	$5.00 \pm$	$0.67 \pm$	4.33 ±	$1.00 \pm$
434		0.89	0.00	0.52	0.00	0.52	1.03	1.03	0.00
KSK.	15.67	$2.00 \pm$	$1.00 \pm$	$0.33 \pm$	$1.00 \pm$	$5.33 \pm$	$1.33 \pm$	$4.67 \pm$	$0.00 \pm$
		1.55	0.52	0.89	0.52	1.37	0.52	0.52	0.52
PK. 386	17.67	$2.00 \pm$	$0.67 \pm$	$1.00 \pm$	$0.67 \pm$	$5.33 \pm$	$1.67 \pm$	$5.67 \pm$	$0.67 \pm$
		0.00	0.00	0.52	0.52	0.52	0.89	1.86	0.52
PS 2	15.67	$2.00 \pm$	$1.00 \pm$	$0.33 \pm$	1.33 ±	5.33 ±	$1.00 \pm$	4.33 ±	$0.33 \pm$
		0.89	0.52	0.52	0.52	1.86	1.55	1.86	1.37

Fo = Fusarium oxysporum	F sp = Fusarium sp	Aa = Alternaria alternata
<i>P</i> sp = <i>Penicillium</i> sp	Af = Aspergillus flavus	A sp = Aspergillus sp
An = Aspergillus nigar	At = Aspergillius terreus	

 Table 10: Percentage frequency of mycoflora associated with germplasm of rice.

Varieties	Fo	F sn	Aa	<i>P</i> sn	Af	A sn	An	At
B. 2000	11 44 +	2.38 ± 3.69	460 + 357	<u> </u>	22.70 +	446 + 348	20.48 +	2.08 ± 3.23
D. 2000	7.73	2.50 ± 5.07	4.00 ± 5.57	3.56	20.89	4.40 ± 5.40	17.90	2.00 ± 5.25
B. 385	15.86 +	4.65 + 3.69	7.86 + 6.19	0.00 ± 0.00	18.08 +	6.41 + 5.03	20.67 +	4.65 + 3.92
21000	2.55				7.08		12.08	
B. Super	$10.00 \pm$	5.00 ± 3.92	7.54 ± 6.42	2.22 ± 3.44	39.68 ±	2.78 ± 4.30	$11.11 \pm$	0.00 ± 0.00
	7.89				36.43		17.21	
B. 515	6.32 ± 0.71	6.61 ± 3.94	3.70 ± 2.87	3.94 ± 3.06	$28.57 \pm$	15.01 ±	$14.02 \pm$	2.38 ± 3.69
					13.60	5.01	12.42	
B. 6129	$10.53 \pm$	3.51 ± 6.44	7.02 ± 7.19	8.27 ± 4.66	$24.81 \pm$	6.52 ± 6.46	$18.30 \pm$	3.51 ± 2.72
	8.15				16.85		10.64	
B. 370	2.56 ± 3.97	2.08 ± 2.72	4.65 ± 3.66	4.42 ± 3.55	$24.84 \pm$	$14.85 \pm$	$24.84 \pm$	3.94 ± 3.06
					9.56	1.91	14.71	
Kisan	4.60 ± 3.57	0.00 ± 0.00	2.38 ± 3.69	2.38 ± 3.69	$23.33 \pm$	$17.96 \pm$	$25.24 \pm$	4.17 ± 6.45
Basmati					9.92	12.44	19.64	
B. 198	$12.09 \pm$	4.62 ± 4.07	4.62 ± 4.07	1.59 ± 2.46	$20.20 \pm$	4.76 ± 7.38	$24.96 \pm$	$10.82 \pm$
	4.31				5.88		8.70	8.56
PS 2	13.33 ±	6.67 ± 1.49	5.00 ± 4.47	8.33 ± 1.49	$25.56 \pm$	6.11 ± 4.79	$17.22 \pm$	1.67 ± 2.58
	2.98				14.17		11.19	
PK. 386	$11.02 \pm$	3.67 ± 3.03	4.44 ± 3.44	3.67 ± 3.03	$22.22 \pm$	9.57 ± 3.06	$24.4 \pm$	3.67 ± 3.03
	9.08				12.41		19.17	
KSK. 434	12.78 ±	6.39 ± 0.22	4.17 ± 3.23	6.39 ± 0.22	27.08 ±	8.33 ± 6.45	16.67 ±	0.00 ± 0.00
	5.61				11.64		12.91	
IR. 6	$13.89 \pm$	4.32 ± 3.52	4.79 ± 3.74	6.54 ± 1.09	$21.03 \pm$	3.98 ± 3.14	$19.49 \pm$	5.73 ± 4.76
1017 100	7.98	1 12 . 2 . 4	5.05 5.05	11.05	17.96	1 (2) 2 71	17.21	1 22 . 2 07
KSK. 133	$10.80 \pm$	4.42 ± 3.64	5.25 ± 5.35	$11.85 \pm$	$23.06 \pm$	4.63 ± 3.71	$21.73 \pm$	1.33 ± 2.07
	4.53	1.50 . 0.46	6.72 . 6.42	3.48	13.70	10 10	18.50	2.24 . 2.60
KSK. 282	$12.36 \pm$	1.59 ± 2.46	6.72 ± 6.42	5.30 ± 0.50	25.58 ±	$12.19 \pm$	$1/./4 \pm$	3.34 ± 2.60
	2.84				13.22	1./1	13.97	

Fo = Fusarium oxysporum P sp = Penicillium sp An = Aspergillus nigar F sp = Fusarium sp Af = Aspergillus flavus At = Aspergillius terreus *Aa* = *Alternaria alternata A* sp = *Aspergillus* sp



Fig. 1: Percentage viability of fine germplasm of rice. Values with different letters show significant difference ($P \leq 0.05$).



Fig. 2: Percentage viability of coarse germplasm of rice. Values with different letters show significant difference ($P \le 0.05$).



Fig. 3: Effect of tetrazolium on husked rice seed at different time intervals.

References

- Almansouri M, Kinet M, Lutts S, 2001. Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf). Plant AOSA Seed Vigor Testing Handbook.
- Martins AB, Marini P, Bandeira JM, 2014. Analysis of seed quality: anon stop evolving activity. *Afr. J. Agric. Res.*, **9**: 49.
- Association of Official Seed Analysts, 1983. Seed Vigor Testing Handbook. Contribution No. 32. Association of Official Seed Analysts. Lincon, NE., USA.
- Association of Official Seed Analysts, 2002. Seed Vigor Testing Handbook Association of Official Analysts, Washington, DC.
- Association of Official Seed Analysts, 2010 and 2014. Tetrazolium testing handbook. Association of Official Analysts, Washington, DC.
- Barnett HL, Hunter BB, 2003. Illustrated Genera of Imperfect Fungi. APS Press.
- Bertolin DC, SA, ME DE, Moreira ER, 2011. Parameters doteste de envelheciment oacelerado para determinacodo vigor de sementes de feijao. *Rev. Bras, Sementes*, **33**: 104-112.
- Daniela VD, Anjos S, Edna UA, Dayana SDM, 2017. Vigor tests to evaluate the physiological quality of corn seeds cv. Sertanejo. *Cienc. Rural*, **47**: 20150705.
- Domsch K, Gams HW, Anderson TH, 2007. Compendium of Soil Fungi, 1st ed., IHW-Verlag und Verlagsbuchhandlung, Postfach 1119, Mu"nchn, pp. 672.
- Duan CX, Wang XM, Zhu AD, Xiao F, 2007. Testing of seedborne fungi in wheat germplasm conserved in the national crop gene bank of China. *Agric. Scien China*, **6**: 682-687.
- Elias SG, Copel LO, McDonald MB and Baalbaki RZ, 2012. Seed testing: Principles and practices. Michigan State Univ. Press, East Lansing. Julio Marcos-Filho Seed vigor testing: an overview of the past, present and future perspective. *Sci. Agric*.**72**: 363-374.
- Gaddeyyaet G, Niharika SP, Bharathi P, Ratna KPK, 2012. Isolation and identification of soil mycoflora in different crop fields at Salur Mandal. *Adv. Appl. Sci. Res.*, **3**: 2020-2026.
- Goettel MS, Inglis GD, 1997 Fungi: Hyphomycetes, pp. 213-249. In: Lacey, L. (Eds). Manuals of technique in insect pathology. Academic Press, New York.
- Humber, RA, 2012. Identification of entomopathogenic fungi, in Manual of Techniques in Insect Pathology, ed. by Lacey LA. Academic Press, San Diego, CA, pp. 151-186.
- Hussain A, 2012. Impact of credit disbursement, areaunder cultivation, fertilizer consumption

and water availability on rice production in Pakistan (1988-2010). *Sarhad J. Agric.*, **28**: 1.

- Ibaim OFA, Umechuruba CI and Arinze AE, 2006. Seed borne fungi associated with seeds of rice (*Oryza sativa*) in storage and from field in Ohaozara and Onicha Local Government Area of Ebonyi State. *World J. Biotechnol.*,**7**: 1062-1072.
- ISTA, 2013. Seed testing rules. International Seed Testing Association, Bassersdorf, Switzterland.
- Kapoor N, Arya A, Siddiqui MA, Hirdesh K, Amir A, 2011. Physiological and biochemical changes during seed deterioration in aged seeds of rice (*Oryza sativa L.*). Am. J. Plant Physiol., 6: 28-35.
- Khajeh-Hosseini M., Powell AA, Bingham IJ, 2003. The interaction between salinity stress and seed vigor during germination of soybean seeds. *Seed Sci. Technol.*, **31**: 715-725.
- Khan AZ, Shah P, Mohd F, Khan H, Amanullah, Perveen S, Nigar S, Khalil SK and Zubair M, 2005. Vigor tests used to rank seed lot quality and predict field emergence in wheat. *Pak. J. Bot.*, **42**: 3147-3155.
- Kubo M, Purevdorj M, 2005. The future of rice production, consumption and seaborne trade: synthetic prediction method. *J Food Dist. Res.*, **36**: 350-359.
- Mahender A, Anandan A, Pradhan SK, 2015. Early seedling vigor, an imperative trait for directseeded rice: an overview on physiomorphological parameters and molecular markers. *Planta*, **241**: 1027-1050
- Martins ABN, Marini P, Bandeira JM, Villela FA, Moraes DM, 2014. Review: Analysis of seed quality: A nonstop evolving activity. *Afr. J. Agric. Res.*, **8**:114-118.
- Milošević M, Milka V, Đura K, 2010. Vigor tests as indicators of seed viability. *Genetika*, **42**: 103-118.
- Okçu G, Kaya MD, Atak M, 2005. Effects of salt and drought stresses on germination and seedling growth of pea (*Pisum sativum* L.). *Turk. J. Agric. For.*, **29**: 237-242.
- Pervez MA, Ayub CM, Khan HA, Shahid MA, Ashraf I, 2009. Effect of drought stress on growth, yield, and seed quality of tomato (*Lycopersicon esculentuml*). *Pak. J. Agric. Sci.*, **46**: 174-178.
- Santos MAO, November ADLC and Filho JM, 2007. Tetrazolium test to assess viability and vigour of tomato seeds. *Seed Sci. Technol.*, **35**: 213-223.
- Singh MK and Kumar S, 2014. Agronomic aspects of zinc biofortification in rice (*Oryza sativa* L.) Prasad. *Proc. Natl. Acad. Sci. India, Sect. B Biol. Sci.*, 84: 613-623.
- Soares VN, Elias SG, Gadotti GI and Villela FA, 2016. Can the tetrazolium test be used as an

alternative to the germination test in determining seed viability of grass species? *Crop Sci.*, **56:** 707-715.

Thu HPT, Thu TN, Thao NDN, Le Minh, K, Do Tan K. (2020). Evaluate the effects of salt stress on physico-chemical characteristics in the germination of rice (*Oryza sativa* L.) in

response to methyl salicylate (MeSA). *Biocatal. Agric. Biotechnol.*, 23, 101470.

Wang ZF, Wang JF, Yong MB, Wang FH and Zhang HS, 2010. Quantitative trait loci analysis for rice seed vigor during the germination stage. J. Zhejiang Univ., 11: 958-964.