



## RESEARCH ARTICLE

# Variability and heterosis of seed vigour traits in hybrid rice (*Oryza sativa* L.)

Shruti Kumari, S. K. Chakrabarty\*, Debashis Paul, Y. Singh, P. K. Bhowmick<sup>1</sup> and A. S. Hari Prasad<sup>2</sup>

## Abstract

The cultivars with high seed vigor are desirable for farmers to establish optimum plant stand under sub-optimal field conditions. The basic physiological processes are required to understand the hybrid vigor phenomenon, particularly for seed vigor and associated characteristics. Limited information on seed vigor in rice hybrids and their parental lines in relation to physical, physiological and biochemical characters are available. Three released rice hybrids and their respective female (A), maintainer (B) and restorer/male (R) lines were assessed for physical, physiological and biochemical traits related to seed vigor. Significant differences were found among the three rice hybrids and its parental lines for 100 seed weight, weight of kernel, husk, endosperm and embryo. Germination percentage, seed vigor index-I and seed vigor index-II were highly significant among female and male lines and hybrids in fresh seed because of potential heterosis in hybrids. Higher activity of reactive oxygen species (ROS) scavenging enzymes such as catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) in hybrids was estimated as compared to that of their parental lines. The study showed the presence of the genetic variability, phenotypic, genotypic and environmental coefficient of variation, heritability, genetic advance and heterosis for seed vigor traits in three rice hybrids and its parental lines. A favorable combination of seed vigor traits would be useful to develop desired variety and hybrids with superior seed vigour for realizing higher grain yield and better adaptability in any condition.

**Keywords:** Rice, hybrid, seed vigour traits, variability, heterosis.

## Introduction

Rice (*Oryza sativa* L.) is one of the important and second largest food grain crops grown in the world in terms of area and production. It is estimated that the population in India is likely to increase to 138.89 crores by the year 2025. To meet the requirements of growing population, about 300 mt of food grains including 130 mt of rice will be required by 2025. India has an average rice productivity of 2.5 t/ha, much lower than the world average, from a total area of 43.79 mha. Environmental factors influence the performance of genotypes, and it has been established that genotype-environment interactions can impact growth. When genotypes are exposed to varying environmental conditions, they are likely to display varying levels of genetic variability with regards to both agronomic and quality traits. The seeds are endowed with genetic, physiological and biochemical properties, all of which are present in the embryo. The embryo and environments largely decide the characteristics of seedlings that later develop into juvenile phases and further into adult phases. One of the most significant applications of genetics in agriculture is the commercial exploitation of hybrid vigour. In rice, heterosis was first reported by Jones (1926) who observed

that some F<sub>1</sub> hybrids had more culms and higher yield than their parents. Earlier, several researchers have reported that through the development of F<sub>1</sub> rice hybrids existence of significant heterosis could be exploited commercially (Chang et al. 1973; Davis and Rutger 1976; Virmani and

---

Division of Seed Science and Technology, <sup>1</sup>Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

<sup>2</sup>ICAR-Indian Institute of Rice Research, Hyderabad 500 030, India

**\*Corresponding Author:** S. K. Chakrabarty, Division of Seed Science and Technology, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India, E-Mail: skchakra\_sst@yahoo.com

**How to cite this article:** Kumari S., Chakrabarty S.K., Paul D., Singh Y., Bhowmick P.K. and Prasad A.S.H. 2023. Variability and heterosis of seed vigour traits in hybrid rice (*Oryza sativa* L.). Indian J. Genet. Plant Breed., **83**(2): 168-175.

**Source of support:** Nil

**Conflict of interest:** None.

**Received:** Dec. 2022 **Revised:** March 2023 **Accepted:** April 2023

---

Edwards 1983; Kim and Rutger 1988). Three principal genetic models namely, dominance, over-dominance, and epistasis have been suggested as explanation for the hybrid phenotype (Crow 1952; Tsaftaris 1995). Depending upon the breeding objectives, both the positive and negative heterosis are useful for crop improvement. To compare the performance of a hybrid based on references, heterosis is expressed in three ways; mid parent, better parent and standard heterosis. Standard heterosis is the most important way to express hybrid performance, which are better than the existing high yielding varieties grown commercially by farmers. The economic success of hybrids might be contributed by seed vigour, an important characteristic of seed quality attributes that determines early, rapid, uniform, germination, emergence and growth of strong seedling in any environmental condition. Seed vigour is an estimate of the successful establishment of seedlings of the variety, hybrids and their parental lines under wide range of conditions experienced in practice. It is determined based on their physical, physiological and biochemical level of heterosis in case of hybrids. Limited literature is available addressing the basis of heterosis which is associated with physical, physiological and biochemical traits influencing seed vigour, particularly in case of hybrids. Hybrid vigour is related to yield, maturity duration and resistance to stress and also governed by seed vigour traits. Keeping these points in view, the present study was undertaken to know the variability and heterosis in rice hybrids and its parental lines for seed vigour related traits.

## Materials and methods

### Seed materials

The seeds of three hybrids and their respective male sterile (A), maintainer (B) and restorer (R) lines of rice were used in this study (Table 1). The A, B and R seeds involved in DRRH 2 and DRRH 3 hybrids were obtained from the ICAR-Indian Institute of Rice Research, Hyderabad. The hybrid seeds of all the three hybrids were produced following the recommended package of practices in the field at ICAR-Indian Agricultural Research Institute, New Delhi during *kharif*, 2018.

### Measurement and analysis of data

Seeds of three hybrids and respective parental lines (A, B and R) were used. Seed or grain in case of rice is called caryopsis. However, we have used the term "seed" in this article as

that is used for reproduction or commercial cultivation. The lemma and palea (together called husk) of a seed is removed to get kernel and it was used for measuring endosperm and embryo length and weight. Observations were recorded on seed and kernel's physical, physiological and biochemical traits. Physical traits comprises of seed length (mm), seed width (mm), kernel length (mm), endosperm length (mm), embryo length (mm), 100 seed weight (g), kernel weight (g) and husk weight (g). Physiological traits include the first count per cent, speed of germination (Maguire 1962), germination percentage (ISTA 2021), seed vigour index-I and seed vigour index-II (Abdul-Baki and Anderson 1973), whereas biochemical traits comprised of electrical conductivity ( $\mu\text{S}/\text{cm}/\text{g}$ ), water soluble sugar ( $\mu\text{g}/\text{ml}$ ) (Dubois et al. 1956), and ROS scavenging enzymes like catalase (Aebi 1984), peroxidase (Castillo et al. 1984) and superoxide dismutase ( $\mu\text{M}/\text{min}/\text{gFW}$ ) (Dhindsa et al. 1981). The heterosis of a character was calculated as the difference between the values of  $F_1$  and that of the average of the two parental lines i.e., mid-parent (MP) and was expressed as percentage increase or decrease over the MP (Virmani et al. 1997).

The data were analysed for variability and mean of the physical, physiological and biochemical traits following the statistical methods as described by Panse and Sukhateme (1985). Heritability, genetic advance, genotypic, phenotypic, environmental variance and heterosis of the seed vigour traits were calculated for hybrids and their parental lines by using R software version 4.2.1.

## Results and discussion

Seedling establishment in desired or predicted number with time is important for successful crop production in diverse crop growing systems. A good number of high yielding varieties and hybrids are being developed and recommended for cultivation. Studies on crop physiology and agronomy attempted to discover the changes the plant breeders made in the new varieties. But, it often failed to address the specific change made to improve crop establishment under varied field conditions. Seed vigour is an important area of research for optimum seedling establishment in rice cultivation in different growing conditions. Hybrid technology has been recognized and found to be a boon for all practical purposes.

### Variability for seed physical traits

In general, hybrid seeds showed intermediate values for seed length, width and weight. However, seed length was higher in DRRH-2 than its parental lines but lower than that of the respective R line for PRH-10 and DRRH-3 (Data not shown). Khush et al.(1988) reported that about 28% of hybrids showing longer seeds, 37% shorter and 38% almost similar to that of the mid-parent value. There is a lower seed set percentage for hybrid seed production than for A line seed production. Therefore, the hybrid seed are likely to get

**Table 1.** Details of hybrids and parental lines used in the study

S. No.	Hybrid	A line	B line	R line
1.	DRRH 2	IR68897A	IR68897B	DR 714
2.	PRH 10	PUSA6A	PUSA6B	PRR 78
3.	DRRH 3	APMS6A	APMS6B	RPHR1005

adequate input and develop well while the A line pollinated by B line may have low inputs for its development that might have expressed in terms of lower seed length. However, all the three hybrids did not show increase in seed length. It was also found that all the three hybrids had higher seed, kernel, and husk weight in comparison to that in the respective parental lines. Higher seed weight and its components in the hybrids could be a feature for hybrid vigour; the endosperm weight being higher and could possibly be the factor for better vigour in hybrid seed. Genetic variability in endosperm and embryo size directly contributed to wheat seed vigor (Nik et al. 2011). Seed size has been reported to influence seedling emergence and establishment in many crop plants (Black 1956). In wheat seed size had no significant effect on germination percentage but positively affected seedling emergence and yield (Zareian et al. 2013). Larger seeds are likely to have larger embryo that help seedling respire at high rate and consequently result in better field emergence (Yang et al. 2015). The larger seed size of hybrid might work in the same way to express better seedling emergence and vigour. Higher endosperm size is correlated with better seedlings growth from our findings (Shruti 2018). However, in wheat embryo size had negligible effect on seedling growth, whereas endosperm size had a large effect (Bremner et al. 1963). Embryo length had the highest phenotypic variance (2.88) and environmental

variance (2.76) but the highest genotypic variance (1.93) and phenotypic variance (1.94) were shown in seed length. The lowest environment variance was observed for kernel length (0.072); phenotypic variance for seed width (0.005) and genotypic variance for endosperm length (-0.30). The genotypic, phenotypic, and environmental coefficients of variance were shown highest for embryo length (43.78, 214.35, and 209.83), respectively. Seed weight (20.27 and 20.29), kernel weight (18.11 and 18.20) and husk weight (40.40 and 41.01) had higher values for the genotypic and phenotypic coefficient of variance (Table 1). The lowest genotypic and phenotypic coefficient of variance was observed for seed width (2.77 and 3.61). In contrast, seed width had the lowest genotypic and phenotypic coefficient of variance, indicating that environmental factors play a more significant role in determining the variation of this trait (Table 2).

Similarly, the phenotypic coefficient of variance, which reflects both genetic and environmental factors, was highest for embryo length, indicating that it is the most variable trait among the measured traits. Seed weight showed the highest heritability (broad sense) (99.8%) and the lowest heritability (4%) observed in embryo length. The heritability of the traits also varied significantly, with seed weight showing the highest heritability. This suggests that genetic factors are the primary determinant of seed weight, making it a desirable

**Table 2.** Range, mean and variability for seed physical and physiological traits in rice parental lines and hybrids

	Range	Mean	F-value	Env. Var.	Gen. Var.	Phen. Var.	ECV	GCV	PCV	H (%)	Gen. Adv.
Physical trait											
SL	7.95-12.37	9.64	*	0.013	1.93	1.943	1.183	14.416	14.464	99.3	2.853
SW	1.95-2.31	2.1	*	0.002	0.003	0.006	2.348	2.771	3.619	58.6	0.092
KL	5.65-8.74	6.99	*	0.072	0.588	0.66	3.844	10.97	11.624	89.1	1.491
ENL	6.30-8.51	6.2	NS	3.318	-0.303	3.015	29.388	8.877	28.015	-10	-0.359
EML	0.05-0.87	0.79	NS	2.769	0.121	2.89	209.83	43.79	214.353	4.2	0.146
SW	1.52-2.97	2.16	*	0.191	0.000	0.192	0.828	20.277	20.293	99.8	0.9
KW	1.14-2.89	1.66	*	0.001	0.091	0.092	1.791	18.111	18.2	99	0.618
HW	0.18-0.81	0.49	*	0.001	0.04	0.041	6.991	40.404	41.01	97.1	0.404
Physiological trait											
FC	72-100	88.11	*	28.44	39.47	67.92	6.05	7.13	9.35	58	9.87
SG	12.62-18.36	15.6	*	0.39	1.91	2.29	4	8.85	9.71	83	2.59
G	86-96	90.81	*	1	6.12	7.12	1.1	2.72	2.94	86	4.73
SVI	870.32-1597.44	1198.54	*	1099.32	37503.32	38602.64	2.77	16.16	16.39	97	393.21
SV II	2.36-5.30	3.97	*	0.02	0.63	0.65	3.65	19.99	20.32	97	1.61

SL= Seed length (mm); SW= Seed width (mm); KL= Kernel length (mm); ENL= Endosperm length (mm); EML= Embryo length (mm); SW= 100 Seed weight (g); KW= Kernel weight (g); HW= Husk weight (g); FC= First count (%); SG= Speed of germination; G= Germination (%); SVI= Seed vigour index-I; SV II= Seed vigour index-II.

\*:significant at  $p=0.05$ ; NS= non-significant; ECV= Environmental Coefficient of Variance; GCV= Genotypic Coefficient of Variance; PCV= Phenotypic Coefficient of Variance; H= heritability

trait to target for genetic improvement in crop breeding programs. Sadras and Slafer (2012) found that in most cereals, namely, wheat, barley, triticale and rice, heritability estimates for seed weight were consistently high across different environments. Genetic advance was observed the highest in seed length (2.85) and endosperm length (-0.35) had the lowest (Table 2). The findings suggest that different physical traits of the embryo have varying degrees of genetic and environmental influences. The genotypic coefficient of variance, which measures the contribution of genetic factors to the total variation of a trait, was highest for embryo length. This suggests that genetic factors have a more significant influence on the variation of embryo length than environmental factors. Li et al. (2022) found that embryo length had a higher heritability and genetic correlation in maize, indicating that it is more under genetic control.

#### ***Variability for seed physiological traits***

All the 12 genotypes were tested for physiological traits and it was found that there were significant differences between hybrids and parental lines for first count (%), speed of germination, germination (%), seed vigour index-I and seed vigour index-II (Data not shown). Our results suggested that hybrids had higher first count (%) than the respective parental lines. This indicates that hybrids have superior initial vigour for early establishment in field condition. It was considered an effective seed vigor assessment method (Menezes et al. 1994). A high variability for first count in seed germination test in rice varieties was reported by (Shenoy et al. 1990). Speed of germination, in a similar way also was found to be superior in hybrids as against the parental lines. It showed how fast most of the seeds are capable to germinate in a given growing condition. First count and seed vigour index-I had the highest environmental variance, phenotypic variance and genotypic variance. Seed vigour index-II was observed lowest environmental, genotypic, and phenotypic variance. The highest genotypic coefficient of variance and phenotypic coefficient of variance was observed for seed vigour index-II (19.99 and 20.32) and the lowest for germination percentage (2.72 and 2.93) (Table 2). The results suggest that seed vigor is a complex trait influenced by genetic and environmental factors, with significant variability observed in different seed vigor indices. In particular, the seed vigor index-II had the highest genotypic coefficient of variance and phenotypic coefficient of variance, indicating that genetic factors have a greater influence on the overall variation of this trait than environmental factors. Zhao et al. (2021) found that seed vigor is under strong genetic control and is influenced by multiple genes. The environmental coefficient of variance was highest for the first count (6.05) and lowest for the germination percentage (1.09). The highest heritability (97%) and genetic advance (393.21) were observed for seed

vigor index-I. Seed vigor index-II had the lowest genetic advance (1.60) and the lowest heritability was shown for the first count (58%) (Table 2). Seed vigor index-I showed the highest heritability and genetic advance, suggesting that genetic factors play a significant role in determining the variation of this trait. The observed environmental variability in seed vigor traits, as indicated by the high environmental variance and environmental coefficient of variance for the first count and seed vigor index-I, highlights the importance of optimizing environmental conditions during seed production and storage to ensure maximum vigor. Previous research has shown that environmental factors such as temperature, moisture, and light can significantly influence seed vigor (Farooq et al. 2021). Thus the genetic and environmental factors influencing seed vigor, can inform breeding programs and management practices to enhance seed quality and maximize crop yields.

#### ***Variability for Seed Biochemical Traits***

Among the seed biochemical traits, seed leachate's electrical conductivity (EC) was measured in terms of  $\mu\text{s}/\text{cm}/\text{g}$  after soaking the seed for 24 hrs. The results showed a significant difference among all the genotypes. In our study all three hybrids estimated lower EC value in the leakage of solutes, indicating higher seed vigor in these as compared to that in the respective parental lines (Table 3). Higher seed longevity in the case of hybrid rice can be inferred through this study. A higher seed storage period with lower EC value of seed in sorghum (Aswathaiah and Sadasivamurthy 1986), soybean (Srivastava and Gill 1975) and black gram (Dharmalingam et al. 1976) have been reported. Further leachate was used to measure water soluble sugar (WSS) in term of  $\mu\text{g}/\text{ml}$  in all the genotypes. The results showed that all genotypes were significantly different for WSS (Data not shown). Increase in the total soluble sugar (TSS) also indicated lower vigor in seeds. In our study a low water soluble sugars also justified the higher hybrid seed vigor. Seed vigor and soluble sugar leaching in rice varieties was reported by Dadlani and Aggarwal (1983).

The role of ROS scavenging enzymes in seed vigour evaluation has been reported by several researchers (Redona and Mackill 1996; Sasahara et al. 1986). ROS being highly reactive, damages macromolecules and inactivates DNA, RNA, proteins and lipids, leading to cellular dysfunctions. The activity of antioxidant enzymes like catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) help removal of ROS as defense mechanism (Schoner and Heinrich, 1990). A higher CAT, POD and SOD activity removes free radicals from seed during ageing or harsh conditions. At the biochemical level hybrid vigor has been studied on different enzyme pattern of hybrids and their parents. The antioxidant enzymes such as POD, SOD, and CAT, have been regarded as the most important defense system to protect the seed from oxidative stress by eliminating the



accumulation of ROS (Foyer and Noctor 2005; Gill and Tuteja 2010).

The antioxidant catalase activity, peroxidase activity and SOD activity was measured in term of  $\mu\text{M}/\text{min}/\text{g}$  FW for all twelve genotypes, including three hybrids. The activity of CAT, POD and SOD varied significantly in all genotypes. Studies on hybrids in cotton indicated high initial growth in vigor and high metabolic activity (Jiang et al. 2006). The heterotic vigor for the antioxidant enzymes (SOD, GR) in hybrid observed in a study could help in understanding the heterosis phenomena for stress tolerance. Higher enzyme activity in the hybrid seed was found in present study, indicating better protection of hybrid seeds from seed deterioration and maintenance of hybrid vigour. Superoxide dismutase (1470.34, 1572.96, and 102.62) exhibited the highest genotypic, phenotypic and environmental variance and lowest for catalase (Table 4). The highest genotypic and phenotypic coefficients of variance are shown for peroxidase (81.16 and 81.16). Catalase (24.97) had the highest environmental coefficient of variance and the lowest for superoxide dismutase (7.65) (Table 3). The results suggest that varying degrees of environmental and genetic influences impact the seed biochemical traits. The study found that superoxide dismutase had the highest genotypic, phenotypic, and environmental variance among the measured traits. This indicates that this trait is significantly affected by both genetic and environmental factors.

On the other hand, catalase had the lowest variance, indicating that this trait is less affected by both genetic and environmental factors. The highest genotypic and phenotypic coefficient of variance was found for peroxidase, indicating that genetic factors have a more significant influence on the variation of this trait than environmental

factors. The highest heritability was showed for catalase (100%) and peroxidase (100%) and the lowest for water-soluble sugar (80%). Superoxide dismutase exhibited the highest genetic advance and the lowest was for catalase (76.37 and 0.02) (Table 3). The highest genotypic and phenotypic coefficient of variance was found for peroxidase, indicating that genetic factors have a more significant influence on the variation of this trait than environmental factors. This finding is consistent with the results of a study by Dhindsa et al. (1981), which found that peroxidase activity is under strong genetic control. The heritability and genetic advance of the traits also varied significantly, with catalase and peroxidase showing the highest heritability and genetic advance. This suggests that genetic factors play a more significant role in determining the variation of these traits. In contrast, water-soluble sugar had the lowest heritability, suggesting that genetic factors less influence this trait. The results provide valuable information on the genetic and environmental factors that influence seed biochemical traits.

#### **Heterosis for seed vigor traits in rice hybrids**

Hybrids are developed and released for cultivation based on hybrid superiority in plant performance-based characters. The increase in seed vigor is not intended in hybrid breeding programme. However, it is interesting to note that the seed vigor traits showed varying levels of standard heterosis values.

#### **Seed physical traits**

A very low level of heterosis was estimated in case of seed physical traits. All three hybrids showed positive heterosis for embryo length, 100 seed weight and kernel weight and negative heterosis for seed width (Table 4). Akita et al. (1986) examined heterosis in  $F_1$  and  $F_2$  rice embryo weight using 200 seeds from each hybrid and showed heterosis

**Table 3.** Range, mean and variability for seed biochemical traits in rice parental lines and hybrids

Parameters	Electrical conductivity ( $\mu\text{S}/\text{cm}/\text{g}$ )	Water soluble sugars ( $\mu\text{g}/\text{ml}$ )	Catalase ( $\mu\text{M}/\text{min}/\text{gFW}$ )	Peroxidase ( $\mu\text{M}/\text{min}/\text{gFW}$ )	Superoxide dismutase ( $\mu\text{M}/\text{min}/\text{gFW}$ )
Range	37.10-159.80	20-77	0.0085-0.0541	0.02-0.22	67.15-190.60
Mean	78.95	53.83	0.03	0.07	132.34
F-value	*	*	*	*	*
Environmental Variance	45.69	61.91	0.00	0.00	102.62
Genotypic Variance	672.15	242.55	0.00	0.00	1470.34
Phenotypic Variance	717.85	304.45	0.00	0.00	1572.97
Environmental Coefficient of Variance	8.56	14.62	24.97	9.69	7.65
GCV	32.84	28.93	38.68	81.16	28.97
PCV	33.94	32.41	38.68	81.16	29.97
Heritability (%) (Broad Sense)	94	80	100	100	93
Genetic Advance	51.68	28.64	0.02	0.11	76.37

\*Significant at  $p=0.05$ ; GCV = Genotypic Coefficient of Variance; PCV = Phenotypic Coefficient of Variance

for embryo weight. Akita (1988) also showed mid-parent heterosis in embryo, endosperm and seedling weight. Ashby (1930, 1932) had also proposed heterosis as an "initial capital theory" based on his observations in maize hybrids. Yamada (1985) reported significant heterosis of  $F_1$  maize embryo. More than 10% heterosis was estimated for embryo length in DRRH-2 and DRRH-3 hybrids. Though PRH-10 showed lower values for embryo length (mm) as compared to the other two hybrids, it showed a higher heterosis value for 100 seed weight, kernel weight and husk weight. In general, the seed length and shape of hybrid grains were reported to be between the two parents (Khush et al. 1988). Negative heterosis was found in PRH-10 for seed length (mm), seed width (mm), kernel length (mm) and endosperm length (mm). DRRH-3 had negative heterosis for seed length, seed width and husk weight. Seed width and husk weight had negative heterosis in DRRH-2 (Table 4). Heterosis for embryo size in mature seeds and developing hybrid embryos over that of the parental lines has been displayed in *Vicia fava* (Dieckmann and Link, 2010) and in developing maize kernels (Jahnke et al., 2010). The seed traits are considered to be controlled by the maternal tissues. The pollen parents do not have any role in its manifestation. Therefore, physical traits in the hybrid seed would be similar to that of the female parental line and heterosis could be negative since male parents possess a superior value for the seed physical traits. Reciprocal hybrids of *Pisum sativum* have proved the maternal effect of seed size difference (Davies, 1975).

### Seed physiological traits

Heterosis for first count (%), germination speed (%), seed

vigor index-I and seed vigor index-II have been investigated to identify the physiological characteristics responsible for superior initial vigor for early establishment in field conditions. All three hybrids had positive heterosis for all physiological traits. First count (%), speed of germination and seed vigor index-I and II showed more than 10% of heterosis (Table 4). Germination percentage showed a low level (3.12-4.44) of heterosis in the fresh seeds. In DRRH-2 and PRH-10 more than 10% heterosis was estimated for the first count and speed of germination. The hybrid seed vigor would definitely have an advantage over a variety with the poor and slow speed of germination. Superiority for seed germination (%) was due to additive/dominant genes present in the two parental lines that were significantly different for seed germination. The role of additive and non-additive type gene action in seedling vigor index and seedling vigor index II inheritance was reported by Akram et al (1993). Seed vigor index-I and Seed vigor index-II showed higher positive heterosis for all three hybrids. As a principle heterosis is manifested better when its parental lines differ widely for the character.

### Seed biochemical traits

The biochemical traits, namely, EC (-1.33 to -38.89) and WSS(-17.95 to -60.96) showed negative heterosis or low values indicating that hybrids have longer storage periods (Table 7). The rate of leakage is an indicator of the aging of seeds. Negative heterosis indicated a low leakage rate in all three hybrids that could cause longer storage without seed deterioration or a lower rate of seed deterioration. Xiao (1979) also reported that hybrid rice's leaf blade and

**Table 4.** Level of heterosis (%) for seed physical, physiological and biochemical traits in rice hybrid seed

Physical traits	Hybrids			Physiological traits	Hybrids			Biochemical traits	Hybrids		
	DRRH-2	PRH-10	DRRH-3		DRRH-2	PRH-10	DRRH-3		DRRH-2	PRH-10	DRRH-3
Seed length (mm)	6.42	-0.30	-1.82	First count (%)	11.27	15.20	1.45	Electrical conductivity* ( $\mu\text{S}/\text{cm}/\text{g}$ )	-2.74	-1.33	-38.89
Seed width (mm)	-0.72	-2.15	-1.15	Speed of germination	10.55	16.13	7.71	Water soluble sugars*( $\mu\text{g}/\text{ml}$ )	-43.27	-60.96	-17.95
Kernel length (mm)	1.50	-3.87	5.32	Germination (%)	4.21	3.12	5.44	Catalase ( $\mu\text{M}/\text{min}/\text{gFW}$ )	34.43	47.36	35.71
Endosperm length (mm)	1.10	-8.58	4.70	Seed vigor index- I	16.73	11.84	30.56	Peroxidase ( $\mu\text{M}/\text{min}/\text{gFW}$ )	80.14	92.56	89.47
Embryo length (mm)	57.89	8.02	44.87	Seed vigor index- II	14.89	10.18	38.05	Superoxide dismutase ( $\mu\text{M}/\text{min}/\text{gFW}$ )	30.56	44.56	39.92
100 seed weight (g)	6.52	27.58	12.69								
Kernel weight (g)	9.50	23.65	17.11								
Husk weight (g)	-4.65	41.07	-6.67								

\* Negative heterosis is in favourable direction

leaf sheath had lower soluble sugar content than inbred check cultivars during its entire growth stage. At the same time the catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) activity showed very high heterosis values in hybrids (Table 4). ROS scavenging enzymes such as CAT, POD and SOD help in removing the free radicals from seed and prevent early ageing.

Heterosis in hybrid rice seeds indicates that the phenomenon occurs widely for physical, physiological and biochemical traits. Positive heterosis was found for embryo length, seed weight, kernel weight, seed vigor index-I and II, catalase and peroxidase. These traits would be useful in selecting the suitable genotype to develop commercial hybrids and varieties with superior seed vigor. A favorable combination of these traits could be the factor for higher seed vigor, resulting in higher grain yield and better adaptability in any condition. Plant breeders can select suitable genotypes based on given traits to develop commercial hybrids. However, the complexity of the heterosis in the case of hybrid seedling establishment, in association with multiple interactions among various factors and pathways (controlled by many genes with possible dominance, over-dominance and/or epistasis), and the temporal progression of plant growth with different processes need to be further studied.

#### Authors' contribution

Conceptualization of research (SKC); Designing of the experiments (SKC, SK); Contribution of experimental materials (PKB, ASH); Execution of field/lab experiments and data collection (SK,YS); Analysis of data and interpretation (SK, DP, SKC); Preparation of the manuscript (SK,SKC).

#### Declaration

The authors declare no conflict of interest.

#### Acknowledgements

The research is part of a M. Sc. Thesis of the first author submitted to Post-Graduate School, ICAR-IARI, New Delhi, India. The first author gratefully acknowledges the Indian Council of Agricultural Research (ICAR) for providing Post-Graduate Scholarship for the study.

#### References

- Abdul-Baki A. A. and Anderson J. D. 1973. Vigor determination in soybean seed by multiple criteria. *Crop Sci.*, **13**: 630-633.
- Aebi H. 1984. Catalase in vitro. *In: Methods in enzymology*, vol. 105. Academic Press: United Kingdom: 121-126.
- Akita S. 1988. Physiological bases of heterosis in rice. *In: Proc. Intern. Symp. on Hybrid Rice* (6 - 10 October, 1986, Changsha, Hunan, China), IRRI, Manila, Philippines: 67-77.
- Akita S., Blanco L. and Virmani S.S. 1986. Physiological analyses of heterosis in rice plant. *Japanese J. Crop Sci.*, **1**: 14-15.
- Akram M., Seshu D. V. and Amin Khan M. 1993. Inheritance of seedling vigour in rice. *Oryza*, **30**: 179-179.
- Ashby E. 1930. Studies in the Inheritance of Physiological Characters: I. A Physiological Investigation of the Nature of Hybrid Vigour in Maize. *Annals Bot.*, **44**: 457-467.
- Ashby E. 1932. Studies on the inheritance of physiological characteristics. II. Further experiments upon the basis of hybrid vigor and upon the inheritance of efficiency index and respiration rate in maize. *Annals Bot.*, **46**: 1001-1032.
- Ashwathiah B. and Sadasivamurthy D. M. 1986. Study on sorghum (*Sorghum bicolor* (L.) Moench) seed quality in storage. *Seeds Farms*, **12**: 9-14.
- Black J. N. 1956. The influence of seed size and depth of sowing on pre-emergence and early vegetative growth of subterranean clover (*Trifolium subterraneum* L.). *Australian J. Agric. Res.*, **7**: 98-109.
- Bremner P. M., Eckersall R. N. and Scott R. K. 1963. The relative importance of embryo size and endosperm size in causing the effects associated with seed size in wheat. *J. of Agric. Sci.*, **61**: 139-145.
- Brown F.B. 1953. Hybrid vigor in rice. *Malaysia Agric. J.*, **36**: 226-236.
- Castillo F. J., Penel C. and Greppin H. 1984. Peroxidase release induced by ozone in *Sedum album* leaves: involvement of Ca<sup>2+</sup>. *Plant Physiol.*, **74**: 846-851.
- Chang T. T., Li C. C. and Tagumpay O. 1973. Genotypic correlation, heterosis, inbreeding depression and transgressive segregation of agronomic traits in a diallel cross of rice (*Oryza sativa* L.) cultivars. *Bot. Bull.*, **14**: 83-93
- Crow J. 1952. Dominance and overdominance. *In: Gowen JW* (ed) *Heterosis*. Iowa State College Press, Ames: 282-297.
- Dadlani M. and Agrawal P. K. 1983. Factors influencing leaching of sugars and electrolytes from carrot and okra seeds. *Scientia Horticulturae*, **19**: 39-44.
- Davies, D. R. 1975. Studies of seed development in *Pisum sativum*: I. Seed size in reciprocal crosses. *Planta*, **124**: 297-302.
- Davis M.D., Rutger I.N. 1976. Yield of F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> hybrids of rice (*Oryza sativa* L.). *Euphytica*, **25**: 587-595.
- Dharmalingam C., Ramakrishnan V. and Ramaswamy K. R. 1976. Viability and vigour of stored seeds of black gram (*Vigna mungo* L. Hepper) in India. *Seed Res.*, **4**: 40-50.
- Dhindsa R.S., Plumb-Dhindsa P.A. and Thorpe T.A. 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Expt. Bot.*, **32**: 93-101.
- Dieckmann, S. and Link W. 2010. Quantitative genetic analysis of embryo heterosis in faba bean (*Vicia faba* L.). *Theor. Appl. Genet.* **120**: 261-270.
- Dubois M., Gilles K. A., Hamilton J. K., Rebers P. T. and Smith F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chem.*, **28**: 350-356.
- Duvick D.N. 1999. Heterosis: Feeding people and protecting natural resources. *In: Genetics and Exploitation of Heterosis in Crops*. American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc. Madison, Wisconsin, USA.
- Farooq S., Onen H., Tad S., Ozaslan C., Mahmoud S.F., Brestic M., Zivcak M., Skalicky M. and El-Shehawi A.M. 2021. The influence of environmental factors on seed germination of *Polygonum perfoliatum* L.: Implications for management. *Agronomy*, **11**: 1123.
- Foyer C. H. and Noctor G. 2005. Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant, Cell Env.*, **28**: 1056-1071.

- Gill S. S. and Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.*, **48**: 909-930.
- ISTA 2021. International Rules for Seed Testing. <https://doi.org/10.15258/istarules.2021.F>.
- Jahnke S., Sarholz B., Thiemann A., Kühn V., Gutiérrez-Marcos J., Geiger H.H., Piepho H.-P. and Scholten S. 2010. Heterosis in early seed development: a comparative study of F<sub>1</sub> days after fertilization embryo and endosperm tissues 6. *Theor. Appl. Genet.*, **120**: 389-400.
- Jiang L., Liu S., Hou M., Tang J., Chen L., Zhai H. and Wan J. 2006. Analysis of QTLs for seed low temperature germinability and anoxia germinability in rice (*Oryza sativa* L.). *Field Crops Res.*, **98**: 68-75.
- Jones J.W. 1926. Hybrid vigor in rice. *J. American Soc. Agron.*, **18**: 424-428.
- Khush G. S., Kumar I, Virmani S. S. 1988. Grain Quality of Hybrid Rice. *In: Proc. Intern. Symp. on Hybrid Rice (6 - 10 October, 1986, Changsha, Hunan, China)* IRRI, Manila, Philippines: 201-215.
- Kim C.H., Rutger I.N. 1988. Heterosis in rice. *In: Hybrid Rice. Proc. Intern. Symp. On Hybrid Rice (6 - 10 October, 1986, Changsha, Hunan, China)* IRRI, Manila, Philippines: 39-54.
- Li X., Wang M., Zhang R., Fang H., Fu X., Yang X., and Li J. 2022. Genetic architecture of embryo size and related traits in maize. *The Crop J.*, **10**: 204-215.
- Maguire J. D. 1962. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. *Crop Sci.*, **2**: 176-177.
- Menezes N. L., De Silveira T. L. D. and Da Pasintoo P. R. 1994. Comparison of methods for rapid evaluation of physiological quality in rice seeds. *Revista Brasileira de Sementes*, **16**: 121-127.
- Nik M. M., Babaean M. and Tavassoli A. 2011. Effect of seed size and genotype on germination characteristic and seed nutrient content of wheat. *Scientific Res. Essays*, **6**: 2019-2025.
- Panse V. G. and Sukhatme P. V. 1985. *Statistical methods for agricultural workers*. ICAR Publication. New Delhi.
- Redona E. D. and Mackill D. J. 1996. Mapping quantitative trait loci for seedling vigor in rice using RFLPs. *Theor. App. Genet.*, **92**: 395-402.
- Sadras V. O. and Slafer G. A. 2012. Environmental modulation of yield components in cereals: Heritabilities reveal a hierarchy of phenotypic plasticities. *Field Crops Res.*, **127**: 215-224.
- Sasahara T., Ikarashi H. and Kambayashi M. 1986. Genetic variations in embryo and endosperm weights, seedling growth parameters and alpha-amylase activity of the germinated grains in rice (*Oryza sativa* L.). *Japanese J. Breed.*, **36**: 248-261.
- Schoner S. and Krause G.H. 1990. Protective systems against active oxygen species in spinach: response to cold acclimation in excess light. *Planta*, **180**: 383-389.
- Shenoy V.V. Dadlani M. and Seshu D.V. 1990. Association of laboratory assessed parameters with field emergence in rice: the nonanoic acid stress as a seed vigour test. *Seed Res.*, **18**: 60-69.
- Kumari S., Chakrabarty S. K., Bhowmick P. K., Singh V. J. and Prasad A. H. 2020. Validation of hybrid rice seed vigour traits using SSR marker (*Oryza sativa* L.). *Indian J. Genet.*, **80**: 204-208.
- Srivastava A.K. and Gill M.K. 1975. Physiology and biochemistry of seed deterioration in soybean. Seedling growth and seed leachate analysis. *Indian J. Expt. Biol.*, **13**: 481-485.
- Tsaftaris S.A. 1995. Molecular aspects of heterosis in plants. *Physiologia Plantarum*, **94**: 362-370.
- Virmani S.S., Edwards I.B. 1983. Current status and future prospects for breeding hybrid rice and wheat. *Adv. Agronomy*, **36**: 145-214.
- Virmani S. S., Viraktamath B. C., Casal C. L., Toledo R. S., Lopez M. T. and Manalo J. O. 1997. *Hybrid Rice Breeding Manual*. International Rice Research Institute, Los Banos, Laguna, Philippines.
- Xiao. 1979. Study on the physiological character of first crop hybrid rice (Sinica). *J. Wuhan University Technol.*, **2**: 24.
- Yamada, M. 1985. Heterosis in embryo of maize, *Zea mays* L. *Bull. National Institute of Agricultural Resources (Japan)*, **1**: 85-98.
- Yang Z., Zheng J., Liu C., Wang Y., Condon A. G., Chen Y. and Hu Y.G. 2015. Effects of the GA-responsive dwarfing gene Rht18 from tetraploid wheat on agronomic traits of common wheat. *Field Crops Res.*, **183**: 92-101.
- Zareian A., Hamidi A., Sadeghi H. and Jazaeri M.R. 2013. Effect of seed size on some germination characteristics, seedling emergence percentage and yield of three wheat (*Triticum aestivum* L.) cultivars in laboratory and field. *Middle-East J. Scientific Res.*, **13**: 1126-1131.
- Zhao J., He Y., Huang S., and Wang Z. 2021. Advances in the identification of quantitative trait loci and genes involved in seed vigor in rice. *Front. Plant Sci.*, **12**: doi: 10.3389/fpls.2021.659307.