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Heterotic Analysis (*Oryza sativa* **L.) in Drought Tolerant Rice Accessions**

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Water-deficit stress tolerance in rice is important for maintaining stable yield, especially under rainfed ecosystem. After a thorough drought-tolerance screening of 200 rice genotypes from DBT, Network Rice Project in our previous study, 19 rice lines were selected for drought tolerance capacity. Six randomly chosen rice accessions out of these 19 rice accessions were further used for creation of F1 Hybrids for heterosis study. The study investigates the heterosis effects on key agronomic traits in rice hybrids, including days to 50% flowering, plant height, number of effective tillers per plant, spikelet fertility, grain yield per plant, and 100-grain weight. Analysis reveals

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diverse heterotic effects across hybrid combinations, with some hybrids exhibiting significant negative heterosis for traits like days to flowering and plant height, indicating potential for early maturation and dwarfism, respectively. Conversely, positive heterosis is observed in traits such as number of effective tillers, spikelet fertility, grain yield, and 100-grain weight, highlighting the potential for enhanced productivity. Notably, certain hybrids consistently display strong positive heterosis across multiple traits, suggesting their suitability for breeding programs aimed at improving overall yield and agronomic performance.

Keywords: Water deficit; standard heterosis; mid parent heterosis; heterobeltosis; line x tester, rice.

1. INTRODUCTION

Drought is the leading threat to agricultural food production, especially in the cultivation of rice, a semi-aquatic plant. Drought tolerance is a complex quantitative trait with a complicated phenotype that affects different developmental stages in plants. Furthermore, to feed the excess population in third-world countries like India, the development of hybrid rice technology might be an inventive genetic approach for narrowing the gap between rice production and demand, as well as augmentation of rice yield up to 15–20% over the current high yielding variety [1,2]. However, rice breeders are facing many challenges in increasing the production of rice [3] due to the decline in rice-growing areas, scarcity of water and labour, and impending intimidations of abiotic and biotic stresses.

In Asia, rice plays a central role in politics, society and culture, directly or indirectly employs more people than any other sector. Farmers must produce good yields without endangering the environment in order to earn a solid living and give the people who eat rice a nutritious, reasonably priced staple food. This is supported by the fact that a robust rice research industry can lower expenses, boost output, and guarantee environmental sustainability. It seems that the most practical and easily adjustable method for raising rice yield levels is hybrid rice technology. In India and outside, a great deal of study is being done on various facets of hybrid rice [4]. The success of the hybrid rice programme depends on the degree of heterosis, which also aids in the identification of possible cross combinations to be used in the conventional breeding programme to create a wide range of variability in the segregating generations [5]. Several pioneer hybrids have demonstrated a yield advantage of about 20% over current three-line hybrids on a commercial scale [4]. Hybrid rice technology has provided a significant contribution to food security and

employment opportunities. Hybrid rice has superior grain yield to inbred rice cultivars and is one of the most significant applications of heterosis in crops [6].

The goal of this work was to find good performing F1 hybrids based on the performance of the estimates of heterosis [7], heterobeltiosis [8], and standard heterosis (comparison of F1 with the best commercial variety). Data from the replicated tests were analysed.

2. MATERIALS AND METHODS

Heterosis study six randomly chosen genotypes from the promising accessions found based on the drought tolerance were taken as testers and five commercial lines were mated in line into tester mating design, and simultaneously two drought tolerant standard checks were taken for the analysis of standard heterosis. Lists of lines testers and standard checks is mentioned in Table 1.

IC514866

Table 1. List of accessions used under Line X Tester mating design

2.1 Statistical Analysis

IR 64 IC460497 Maheshwari IC463878

Heterosis was calculated using the overall average of each hybrid over the replication for each trait. The percent of heterosis for each trait in F_1 was worked out by applying the method proposed by Liang et al. [9] in relation to mid parent (average heterosis), better parent (heterobeltiosis) and standard checks (standard heterosis).

2.2 Average/Mid-parent Heterosis

It was estimated as percent deviation of a mean value of F_1 from its mid-parental value.

Average heterosis =
$$
\frac{\overline{F1} - \overline{MP}}{\overline{MP}} X 100
$$

Where,

 $\overline{F1}$ = Mean performance of hybrid \overline{MP} = Mean performance of both parent involved in the crosses

2.3 Heterobeltiosis

It was estimated as the deviation of hybrid mean from the mean of better parent.

Heterobeltiosis =
$$
\frac{\overline{F1} - \overline{BP}}{\overline{BP}}
$$
X 100

Where,

$$
\overline{BP}
$$
 = Mean performance of better parent

2.4 Standard Heterosis

It is also known as useful heterosis and was estimated as the percent deviation of hybrid mean from the mean of standard check.

Average heterosis =
$$
\frac{\overline{F1} - \overline{SC}}{\overline{SC}} X 100
$$

Where,

 \overline{SC} = Mean performance of the standard check

The significance of different types of heterosis was assessed by adopting 't' test as suggested by Snedecor and Cochran [10]:

't cal' for mid-parameter
$$
= \frac{F1 - MP}{\sqrt{3EMS/r}}
$$

't cal' for heterobeltiosis = $\frac{F1-BP}{\sqrt{(2EMS/r)}}$

't cal' for standard heterosis = $\frac{F1 - SC}{\sqrt{(2EMS/r)}}$

Where,

EMS = Error mean sum of squares in the ANOVA table

r = Number of replications

After finding the 't' calculated value it was compared to the tabulated 't' value at corresponding error degrees of freedom for each trait.

The significance of estimated heterosis levels were evaluated using the t test as described by Turner [11]. Estimated t values were compared with two tailed tabular t values with corresponding error degrees of freedom at the 95 and 99% confidence intervals [3,12].

Gramaje [3] Heterosis and combining ability analysis in CMS hybrid rice. *Euphytica*, *216*, 1- 22.

Turner [11] a study of heterosis in Upland cotton II. Combining ability and inbreeding effects.

Kumar [12] Estimation of heterosis for earliness and certain growth characters in rice (Oryza sativa L.). *Plant Archives*, *20*(2), 1429-1432.

3. RESULTS AND DISCUSSION

3.1 Heterosis Studies

Investigations on heterosis provides fundamental information regarding the utility of the cross combinations and its use for commercial exploitation. The magnitude of heterosis for grain yield, yield components and quality traits depends to a large extent on genetic variation, genetic base and adaptability of parents. The presence of significant amount of non-additive gene action is a prerequisite for the commercial exploitation of heterosis in rice [13].

A program to produce hybrid population may be initiated for a number of reasons. A partial listing of these would include existence of a significant amount of dominance variance, a requirement for high degree of uniformity in the harvested product, a need for flexibility in the program and the availability of cytoplasmic sterility. The demonstration of heterosis in crop is not adequate justification for the establishment of a program to produce hybrids. The existence of heterosis shows two things, some degree of genetic diversity between parents and some degree of dominance. Heterosis can arise when many loci are involved, if for each locus, the heterozygote is slightly super to the mid-parental value [14]. The existence of therefore, provides no guide as to the degree of dominance. Such information must be derived from specifically designed experiments.

The Heterosis over mid parent (Relative heterosis), over better parent (heterobeltiosis) and over standard check (standard heterosis/ useful heterosis) was estimated for all the

characters under study. The estimates of mid parent, better parent and standard heterosis are given in Table 2.

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**Significant at p=0.05% level, **Significant at p=0.01% level*

**Significant at p=0.05% level, **Significant at p=0.01% level*

3.2 Days to 50% Flowering

The relative heterosis for this trait ranged from - 21.33 % (RRF 140 X IC458657) to 20.43 % (Swarna X IC516693). The highest significant negative heterosis over mid parent recorded by cross RRF 140 X IC458657 (-21.33 %) followed by RRF 140 X IC514866 (-15.37 %). The heterobeltiosis ranged from –25.25 % (RRF 140 X IC458657) to 4.98% (Swarna X IC516693). The highest significant negative heterosis over better parent recorded by cross RRF 140 X IC458657 (-25.25 %) followed by RRF 140 X IC514866 (-22.26 %), RRF 140 X IC516693 (- 21.26), RRF 140 X IC460497 (-18.27), RRF 140 X IC463878 (-17.28) and RRF 140 X IC206758 (- 16.61). The standard heterosis (over DRR DHAN 42) ranged from -2.17% (RRF 140 X IC458657) to 21.74 % (Swarna X IC516693). Heterosis in both negative and positive direction for days to flowering has also been reported by by Premkumar et al. [15] and Awad-Allah, [16].

Negative heterosis is desirable for days to flowering because this will make the hybrids to mature earlier as compared to parents. Testers IC458657 showed highest significant negative estimates for mid and better parent heterosis. Tester IC458657 showed significant negative value for all heterotic estimates. IC458657 with IR 64 showed heterobeltiosis and relative heterosis in negative direction. It can also be used for developing early maturing hybrids.

The analysis of days to 50% flowering highlights a diverse range of heterosis values across hybrid combinations, indicating significant potential for influencing flowering time in breeding programs. Notably, hybrids involving RRF 140 consistently exhibited substantial negative heterosis, particularly notable in comparisons with both mid and better parent values. This suggests a genetic predisposition towards early flowering, making RRF 140 a promising candidate for developing early maturing hybrids. Tester IC458657 consistently demonstrated significant negative estimates across various heterotic measures, further underscoring its suitability for facilitating early maturation traits in hybrids. Additionally, the preference for negative heterosis aligns with the objective of achieving earlier maturity compared to parental lines.

3.3 Plant Height (cm)

The mid parent heterosis ranged from -19.42 % (RRF 140 X IC514866) to 35.50 % (Dagad desi X IC463878). Highest negative heterosis in plant height was obtained by cross RRF 140 X IC514866 (-19.42). The heterobeltiosis ranged from -26.43 % (RRF 140 X IC206758) to 22.20 % (Dagad desi X IC463878). Highest negative heterobeltosis in plant height was obtained by cross RRF 140 X IC206758 (-26.43). The standard heterosis (over IGKV R2) ranged from - 15.21% (IR64 X IC206758) to 30.29 % (Dagad desi X IC463878). Highest negative standard heterosis in plant height was obtained by cross IR64 X IC206758 (-15.21). The standard heterosis (over DRR DHAN 42) ranged from - 0.29 % (IR64 X IC206758) to 53.22 % (Dagad desi X IC463878). Among hybrids, none of them showed significant negative heterosis and nineteen hybrid showed significant positive heterosis for this trait.

Negative heterotic effects indicating that the hybrids were shorter than their mid parents and positive heterotic effects showed that hybrids were taller with their mid parents. Negative heterosis is desirable for plant height because this will make the hybrids to give more reproductive growth compared to parents. Testers IC514866 and IC206758 showed highest significant negative estimates for mid and better parent heterosis. Tester IC206758 showed significant negative value for all heterotic estimates over IGKV R2. Tester IC206758 with RRF 140 showed heterobeltiosis, relative heterosis and standard heterosis over IGKV R2 in negative direction. It can also be used for developing dwarf hybrid.

Similar results have also been reported by Sahu et al. [17], Borah et al. [18], Premkumar et al. [15], Thorat et al. [19], Kumar et al. [12], Awad-Allah, [16], El-Mowafi et al. [20] and Meena et al. [21]

A broad range of heterosis effects are shown by analysing plant height attributes across different hybrid combinations, with both positive and negative values affecting plant stature in comparison to parental lines. Notably, throughout several comparisons, hybrids including RRF 140 continuously showed significant negative heterosis, suggesting a tendency towards lower height in relation to the mid and higher parent values. This implies the possibility of producing dwarf hybrids, which would be especially interesting in crossings including the tests IC514866 and IC206758. Tester IC206758 is especially notable because it has the potential to be used in the breeding of dwarf varieties, as it consistently showed significant negative estimations across heterotic measurements. On the other hand, hybrids such as Dagad desi X IC463878 showed a strong positive heterosis, suggesting a tendency towards greater height in

comparison to the parental lines. This might be useful for some breeding goals.

3.4 Number of Effective Tillers per Plant

The mid parent heterosis ranged from -33.33 % (Dagad desi X IC514866) to 84.31 % (Swarna X IC206758). Highest positive heterosis in number of effective tillers per plant was obtained by cross Swarna X IC206758 (84.31). The heterobeltiosis ranged from -50.00 % (Dagad desi X IC514866) to 74.07 % (Swarna X IC206758). Highest positive heterosis in number of effective tillers per plant was obtained by cross Swarna X IC206758 (74.07). The standard heterosis (over IGKV R2) ranged from -51.61 % (Maheshwari X IC460497) to 58.06 (Dagad desi X IC206758). Highest positive heterosis in number of effective tillers per plant was obtained by cross Dagad desi X IC206758 (58.06). The standard heterosis (over DRR DHAN 42) ranged from -54.55 % (Maheshwari X IC460497) to 48.48 % (Dagad desi X IC206758). Highest positive heterosis in number of effective tillers per plant was obtained by cross Dagad desi X IC206758 (48.48).

There is considerable potential for altering tillering capacity in breeding programmes, as evidenced by the examination of the number of effective tillers per plant across various hybrid combinations, which demonstrates a wide variety of heterosis effects. Notably, relative to parental lines, a number of hybrids—most notably Swarna X IC206758—exhibited highly significant positive heterosis, indicating a notable increase in tillering. This points to a possible way to boost grain yield by producing more tillers. On the other hand, hybrids such as Dagad desi X IC514866 showed a notable decrease in tiller numbers in comparison to their parental lines, as evidenced by their strong negative heterosis. The preference for positive heterosis over better parent and standard checks aligns with the objective of maximizing tiller production for improved crop yield. Additionally, the consistent demonstration of significant heterosis effects across different comparison standards underscores the robustness of these findings, providing valuable insights for breeders aiming to optimize tillering traits in crop hybrids.

High heterosis for more number of panicles per plant has been reported by Karpagam and Kalaiyarasi, [22], Premkumar et al. [15], Thorat et al. [19], Venkatesan et al. [23], Kumar et al. [12] and El-Mowafi et al. [24].

3.5 Number of Filled Grains per Panicle

The mid parent heterosis ranged from -43.59 % (Dagad desi X IC206758) to 42.12 % (IR64 X IC514866. Highest positive heterosis in number of filled grains per panicle s was obtained by cross IR64 X IC514866 (42.12). The heterobeltiosis ranged from -47.23 % (Dagad desi X IC206758) to 31.60 % (IR64 X IC514866). Highest positive heterosis in number of filled grains per panicles was obtained by cross IR64 X IC514866 (31.60). The standard heterosis (over IGKV R2) ranged from -48.38 % (Dagad desi X IC206758) to 18.77 % (IR64 X IC514866). Highest positive heterosis in number of filled grains per panicle s was obtained by cross IR64 X IC514866 (18.77).

The number of number of filled grains per panicle directly contributes to grain yield hence positive heterotic effect would be highly desirable. In the present study, more number of number of filled grains per panicle is closely associated with high grain yield per plant resulting high productivity. Therefore, the main interest is to find out the cross combinations with more number of long and heavy panicle bearing tillers. The tester IC514866 with line IR 64 recorded higher values of heterotic expression for better parent, mid parent and check IGKV R2. Virmani et al. [25,26] reported that heterosis in grain yield was primarily due to increased number of spikelets per panicle further supported by Patel et al. (1994) and Reddy (1996) that confirms the present trend in these traits.

Analysing the amount of filled grains per panicle in a variety of hybrid combinations shows strong heterosis effects, indicating a great deal of potential for affecting grain output in breeding programmes. Some hybrids, like IR64 X IC514866, showed considerable positive heterosis, meaning that the number of filled grains was higher than in the parental lines. Other hybrids, like Dagad desi X IC206758, showed significant negative heterosis, indicating that the number of filled grains was lower. Given that they directly affect grain production and overall productivity, this emphasises the significance of choosing hybrid combinations with positive heterotic effects. IC514866 showed stronger heterotic expression across many comparative standards, especially when combined with line IR64, highlighting its potential for generating hybrids with better grain-filling traits. This is consistent with other research that suggests an increase in spikelets per panicle is

the primary cause of heterosis in grain production. This emphasises the significance of choosing hybrids with improved panicle features in order to achieve higher productivity.

Significant positive heterosis for number of number of filled grains per panicle s per panicle was reported by Devi et al. [27], Premkumar et al. [15] and Ramesh et al. [28], Meena et al. [21] and El-Mowafi et al. [24].

3.6 Spikelet Fertility (%)

The mid parent heterosis ranged from -52.89 % (Dagad desi X IC206758) to 15.62 % (RRF 140 X IC463878. Highest positive heterosis in spikelet fertility per panicle was obtained by cross RRF 140 X IC463878 (15.62). The heterobeltiosis ranged from -57.74 % (Dagad desi X IC206758) to 5.34 % (IR64 X IC516693). The standard heterosis (over IGKV R2) ranged from -54.32 % (Dagad desi X IC206758) to 11.97 % (RRF 140 X IC514866). Highest positive heterosis in spikelet fertility per panicle was obtained by cross RRF 140 X IC514866 (11.97). The standard heterosis (over DRR DHAN 42) ranged from -54.49 % (Dagad desi X IC206758) to 11.54 % (RRF 140 X IC514866). Highest positive heterosis in spikelet fertility per panicle was obtained by cross RRF 140 X IC514866 (11.54).

The evaluation of spikelet fertility percentage in different hybrid combinations shows a broad range of heterosis effects, suggesting a substantial potential to affect the success of reproduction in breeding programmes. Significant negative heterosis was seen in some hybrids, such as Dagad desi X IC206758, indicating a drop in fertility, while significant positive heterosis was seen in some hybrids, such as RRF 140 X IC463878 and RRF 140 X IC514866, suggesting an increase in spikelet fertility relative to parental lines. This emphasises how crucial it is to choose hybrid combinations that have favourable heterotic effects because they have a direct impact on yield potential overall. Among the comparative standards, RRF 140 X IC514866 had the highest positive heterosis continuously, indicating that it may have the ability to increase spikelet fertility and ultimately crop productivity.

Most of the hybrids had negative heterosis due to the problem of spikelet sterility, as reported by Virmani et al. [26]. Standard heterosis of both positive and negative nature was observed by Panwar et al. [29] whereas; similar nature for heterobeltiosis was reported by and Belhekar et al. [30]. Positive heterosis over better parent and standard variety was reported by Virmani et al. [25] they concluded that heterosis in grain yield was primarily due to increased number of filled grains per panicle s per panicle. Similar result was also observed by Devi et al. [27], El-Mowafi et al*.* [24].

3.7 Grain Yield per Plant (g)

The mid parent heterosis ranged from -64.63 % (Swarna X IC458657) to 38.03 % (IR64 X IC463878). Highest positive heterosis in grain yield per plant was obtained by cross IR64 X IC463878 (38.03). The heterobeltiosis ranged from -66.28 % (Swarna X IC458657) to 32.43 % (IR64 X IC463878). Highest positive heterosis in grain yield per plant was obtained by cross IR64 X IC463878 (32.43). The standard heterosis (over IGKV R2) ranged from -60.81 % (Swarna X IC458657) to 37.84 % (Swarna X IC206758). Among hybrids, fifteen hybrids showed significant positive standard heterosis while eleven hybrids showed significant negative standard heterosis for this trait. Highest positive heterosis in grain yield per plant was obtained by cross Swarna X IC206758 (37.84). The standard heterosis (over DRR DHAN 42) ranged from - 62.82 % (Swarna X IC458657) to 30.77 % (Swarna X IC206758). Highest positive heterosis in grain yield per plant was obtained by cross Swarna X IC206758 (30.77).

Grain yield is a complex trait that is multiplicative end product of several attributes of yield. Hybrid showing high heterosis for grain yield per plant, also manifested heterotic effects for productive tillers per plant, panicle length, fertile grains per panicle.

There is a great deal of opportunity for increasing yield in breeding programmes when the examination of grain yield per plant across various hybrid combinations shows a wide variety of heterosis effects. A significant increase in grain yield compared to parental lines was indicated by several hybrids that showed significant positive heterosis, including IR64 X IC463878 and Swarna X IC206758. In order to optimise yield potential, this emphasises how crucial it is to choose hybrid combinations with favourable heterotic effects. Hybrids such as Maheshwari X IC514866 have been seen to exhibit constant high positive heterosis when compared to different standards, indicating their potential for higher yield performance.

Furthermore, the fact that other yield-related characteristics like as the number of productive tillers per plant and the number of viable grains per panicle are correlated with grain yield emphasises the multifactorial nature of grain production and the necessity of comprehensive breeding methods that target various yield components. For breeders hoping to create crop hybrids with improved agronomic performance and high yields, these results provide insightful information.

Increased grain yield in rice due to various component traits as observed in the present investigation is in close conformity the finding observed by the other workers Anis et al. [20], Devi et al. [24], Karpagam and Kalaiyarasi, [22], Thorat et al[19], Venkatesan et al. [23] and El-Mowafi et al. [24].

3.8 100 Grain Weight (g)

The mid parent heterosis ranged from -12.28 % (RRF 140 X IC206758) to 24.84 % (Dagad desi X IC516693). Highest positive heterosis in 100 seed weight was obtained by cross Dagad desi X IC516693 (24.84). The heterobeltiosis ranged from -19.53 % (RRF 140 X IC206758) to 14.94 % (Dagad desi X IC516693). Highest positive heterosis in 100 seed weight was obtained by cross Dagad desi X IC516693 (14.94). The standard heterosis (over IGKV R2) ranged from - 11.76 % (RRF 140 X IC206758) to 21.18 % (IR64 X IC458657). Highest positive heterosis in 100 seed weight was obtained by cross IR64 X IC458657 (21.18). The standard heterosis (over DRR DHAN 42) ranged from -16.29 % (RRF 140 X IC206758) to 14.96 % (IR64 X IC458657). Highest positive heterosis in 100 seed weight was obtained by cross IR64 X IC458657 (14.96).

Significant heterosis effects are revealed when 100-grain weight is compared between different hybrid combinations, underscoring the possibility of improving this crucial yield factor in breeding programmes. Notably, a number of hybrids including IR64 X IC458657 and Dagad desi X IC516693 showed significant positive heterosis, suggesting a notable increase in seed weight in comparison to parental lines. Grain weight plays a major role in total yield, which emphasises how important it is to choose hybrid combinations with positive heterotic effects to maximise yield potential. The trait hundred grain weight is an important yield component in the final grain yield, as the bold grained varieties normally out grain yield the other types. In the present study

positive significant values are reported which were in agreement with the earilier findings by Rahimi et al. [31], Krishna et al. (2011), Pratap et al. [32] and Latha et al. [33].

4. CONCLUSION

In conclusion, the study underscores the importance of heterosis effects in shaping rice hybrid performance across various agronomic traits. The findings suggest avenues for targeted breeding efforts to enhance traits crucial for yield and productivity. Notably, hybrids exhibiting consistent positive heterosis across multiple traits hold promise for improving overall crop performance. Furthermore, the identification of hybrids with significant negative heterosis for specific traits presents opportunities for developing specialized varieties, such as early maturing or dwarf hybrids. Overall, the insights provided by this research offer valuable guidance for rice breeders seeking to optimize hybrid combinations for enhanced agronomic performance and yield potential.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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