Estimation of heterotic effects under water deficit conditions in rapeseed genotypes (Brassica napus L.)

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Citation

Abstract
Water stress is one of the primes constrains for numerous field crop production including rapeseed. The development of water stress tolerant rapeseed varieties is considered as an efficient and effective approach to face the challenges of water scarcity. However, to carry out genetic analysis such as, heterosis help to evolve high yielding crop varieties for stress breeding. In this context, six parents and fifteen F1 hybrids were grown against water stresses during growing season 2019-20 under randomized complete block design in factorial arrangements with three replications and two treatments, such as, (i) control (4 irrigations applied at four main growth stages i.e., stem growth, flowering, silique formation and maturity) and (ii) water stress at silique formation (2 irrigations applied at two main growth stages i.e., stem growth and flowering) for the estimation of genetic parameters. The obtained results of the mean squares showed that genotypes treatments and their interactions (genotype × treatment) were significantly differences (P≤0.05) for majority of the studied characters. Whereas the maximum heterotic effects over better parent for seed yield plant1 and oil content, under normal and water stress conditions reported by the hybrids Rohi Sarson × Punjab Sarson, Hyola-401 × Punjab Sarson and Hyola-401 × NARC, respectively. Whereas highest heterotic effects over mid parent for seed yield plant1 and oil content, reported by the F1 hybrids such as, Rohi Sarson × Punjab Sarson, Hyola-401 × Punjab Sarson and Hyola-401 × NARC Sarson under both growing conditions. All these results demonstrated that breeding materials used in the present research has worth to be used in future rapeseed breeding programs.

Keywords: Brassica napus; Heterosis; Oil content; Seed yield; Water scarcity

Introduction
Rapeseed (Brassica napus) is an oilseeds crop, generally grown in subtropical and tropical countries [1]. After palm and soy oil, Rapeseed came on 3rd in list to produce edible oil at the global level. It has been found an
ideal source of vegetable oil and as a successful mediator against cardiovascular diseases and insulin resistance [2, 3]. The seeds of Brassica napus tend to have 40-46% oil in its seeds. During 2017-18, the availability of total edible oil in Pakistan was 3.623 million tonnes, while only 0.431 million tonnes i.e., 12% of total availability was locally produced and 88% was imported by spending 320.893 billion rupees. During 2018-19, 237 million hectares of mustard and rapeseed were grown with 302 million tons of production, while during 2019-20, 349 million hectares of mustard and rapeseed were cultivated with 302 million tons of production [4].

Improvement of rapeseed genotypes under drought stress has rarely been included in breeding programs [5]. There are quantitative differences in drought tolerance in collections of inbred rapeseed lines, so it is possible to improve genetically drought tolerance [6]. Pakistan is facing problems in many crops due to water stress [4]. Water deficit affects the production, yield and development of the crops, which are most significant stresses for crops of agricultural relevance [7]. A significant change in the seed yield can occur due to water stress that occurs at any time during reproductive development. The potential of rapeseed crop in terms of seed yield depends on the stages, while the reproductive stage of crop is most vulnerable to water stress. Severe stress declines reproductive growth and stress during the flowering or maturity stage induces substantial loss of yield [8, 9]. The maximum decrease in seed yield was achieved once water stress imposed at two stages i.e. flowering and pod development phases of plants [10].

Considering heterosis, it is a genetic phenomenon that describes the positive or negative results of the first generation of hybrids. It can only occur in the first hybrid generation, where the interest of a specific function (yield) exceeds that of the better parent, while this phenomenon cannot be maintained throughout generations [11]. Thus far, the heterotic phenomenon has not been clarified entirely, because its manifestations are too complex for a single stipulation to define [12]. Heterosis can result in differences in the genome structure, especially in the distribution and presence in the crossed inbred lines, which possess certain genes in the given gene family, since last century, hybrid vigor is being used progressively in crop production to produce higher quality, more robust and competitive hybrid crops [13]. According to [14, 15], the effective heterosis for rapeseed has been recorded within spring type, winter type and semi-winter type.

**Materials and Methods**

The current experiment was conducted at Botanical Garden, Sindh Agriculture University, Tandojam during the rabi season 2019-20. In this respect, six parents, such as (Con-1, Hyola-401, Rainbow, Rohi Sarson, Punjab Sarson and NARC Sarson) and their corresponding fifteen F₁ hybrids of rapeseed genotypes (*Brassica napus* L.) were sown in a randomized complete block design in factorial arrangements with three replications and two treatments. The treatments were set as T₁ (Control = 4 irrigations at four main stages i.e. stem growth, flowering, silique formation and maturity); T₂ and (2 irrigations at two main stages i.e. stem growth and flowering, whereas two irrigations at silique formation and maturity was stopped for imposing water stress. A total of Ten traits were investigated, such as Plant height (cm), Branches plant⁻¹, Silique length (cm), Seed yield silique⁻¹ (g), seed yield plant⁻¹, seed index (1000-seed weight, g), chlorophyll SPAD, relative water content (%), oil content (%) and protein content (%). The key focus in this research was to carry out a genetic analysis (heterosis) in water stress conditions of water deficit.
Data analysis
The values of mean squares were acquired according to [16]. The mid-parent heterosis (MPH) and better parent heterosis (BPH) were calculated by the formula as: MPH = \([F_1-(P_1+P_2)/2] / ((P_1 + P_2)) \times 100\%\) and BPH = \((F_1-HP)/HP \times 100\%\), where \(F_1\) is the value of \(F_1\) crosses, \(P_1\) and \(P_2\) are the phenotypic value of parents, HP is the phenotypic value of higher value parent.

Meteorological parameters (Rainfall and temperature)
Meteorological data including rain and temperature was collected from Agro-Meteorological Data Regional Agromet Centre Tandojam. Monthly wise data is given in (Table 1). According to the meteorological data no rain was recorded during the month of October 2019 to March 2020.

Table 1. Total rain and temperature recorded in different growing seasons

<table>
<thead>
<tr>
<th>Months</th>
<th>Total rain (mm)</th>
<th>Temperature</th>
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<tr>
<td></td>
<td>Total</td>
<td>Means</td>
</tr>
<tr>
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<tr>
<td>October-2019</td>
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<tr>
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<td>0.0</td>
</tr>
<tr>
<td>March-2020</td>
<td>0.0</td>
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</tbody>
</table>

Table 2. Mean squares of various traits of parents and \(F_1\) hybrids of rapeseed genotypes under control and water stress conditions

<table>
<thead>
<tr>
<th>SOV</th>
<th>Degrees of Freedom</th>
<th>Replication</th>
<th>Genotypes</th>
<th>Treatments</th>
<th>G × T</th>
<th>Parents</th>
<th>(F_1) hybrids</th>
<th>P vs. C</th>
<th>Errors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characters</td>
<td></td>
<td>2</td>
<td>20</td>
<td>1</td>
<td>20</td>
<td>5</td>
<td>14</td>
<td>1</td>
<td>82</td>
</tr>
<tr>
<td>Plant height</td>
<td>4.23</td>
<td>1967.70**</td>
<td>1578.34**</td>
<td>12.08**</td>
<td>1882.22**</td>
<td>436.07**</td>
<td>23837.93**</td>
<td>8.93</td>
<td></td>
</tr>
<tr>
<td>Branches plant (^1)</td>
<td>0.2965</td>
<td>12.5026**</td>
<td>40.7893**</td>
<td>0.1087**</td>
<td>3.35111**</td>
<td>1.8832**</td>
<td>206.9309**</td>
<td>0.1123</td>
<td></td>
</tr>
<tr>
<td>Silique length</td>
<td>0.0625</td>
<td>40.3658**</td>
<td>37.1206**</td>
<td>0.1102*</td>
<td>2.90809**</td>
<td>10.8675**</td>
<td>640.6306**</td>
<td>0.0628</td>
<td></td>
</tr>
<tr>
<td>Seed yield silique (^1)</td>
<td>1.388</td>
<td>0.00451**</td>
<td>0.01284**</td>
<td>2.584**</td>
<td>0.00068**</td>
<td>5.789**</td>
<td>0.07867**</td>
<td>1.910</td>
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<tr>
<td>Seed yield plant (^1)</td>
<td>0.198</td>
<td>126.145**</td>
<td>355.018**</td>
<td>0.579**</td>
<td>2.6273**</td>
<td>22.938**</td>
<td>2188.617**</td>
<td>0.357</td>
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</tr>
<tr>
<td>Seed index</td>
<td>0.0154</td>
<td>14.9338**</td>
<td>19.4975**</td>
<td>0.2935**</td>
<td>0.01788**</td>
<td>2.9177**</td>
<td>257.7393**</td>
<td>0.0202</td>
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<tr>
<td>Chlorophyll SPAD</td>
<td>1.119</td>
<td>133.884**</td>
<td>729.894**</td>
<td>2.769**</td>
<td>62.836**</td>
<td>17.043**</td>
<td>2124.901**</td>
<td>1.347</td>
<td></td>
</tr>
<tr>
<td>Relative water content</td>
<td>0.031</td>
<td>290.521**</td>
<td>887.646**</td>
<td>2.899**</td>
<td>108.893**</td>
<td>13.249**</td>
<td>5080.487**</td>
<td>2.819</td>
<td></td>
</tr>
<tr>
<td>Oil content</td>
<td>0.0225</td>
<td>20.5280**</td>
<td>67.9067**</td>
<td>0.1591**</td>
<td>0.9138**</td>
<td>2.7492**</td>
<td>367.5029**</td>
<td>0.0128</td>
<td></td>
</tr>
<tr>
<td>Protein content</td>
<td>0.0081</td>
<td>57.6230**</td>
<td>7.0763**</td>
<td>0.2246**</td>
<td>63.6049**</td>
<td>37.1904**</td>
<td>313.771**</td>
<td>0.0216</td>
<td></td>
</tr>
</tbody>
</table>

Results
Mean squares
The mean squares from analysis of variances for treatments, genotypes, crosses and parents vs. crosses (P vs. C) showed significantly differences (p≤0.05) for maximum of the parameters, such as plant height, branches plant \(^1\), silique length, seed yield silique \(^1\), seed yield plant \(^1\), seed index, Chlorophyll SPAD, relative water content, oil content and protein content, whereas genotype × treatment interactions were also significantly different for majority of the studied traits like as, Silique length, seed index, Chlorophyll SPAD, oil content and protein content, however breeding material specified highest genetic variability (Table 2).
Heterosis
The heterotic effects for different parameters under normal and water deficit conditions are given in (Fig. 1-10).

Plant height
Under normal and water deficit conditions, all 15 F1 hybrids presented positive heterosis for mid and better heterosis. The highest positive effects over mid parent were disclosed by same hybrid Hyola-401 × NARC Sarson under normal (28.58%) and water deficit conditions (28.10%), however the lowest positive effects over mid parent were (11.26% under control and 10.32% under water stress conditions) recorded by Rainbow × Punjab Sarson. Under control (14.61%) and water stress conditions (20.81%), the maximum positive direction better parent heterosis were noted in hybrid Con-I × Rohi Sarson, whereas minimum positive better parent effects under both growing conditions (normal = 9.75% and water stress = 3.79%) were demonstrated by Hyola-401 × Rainbow (Fig. 1).

Branches plant
The significant and positive results of heterotic effects over mid and better parent were obtained from all the 15 hybrids under non stress and water stress conditions. The maximum positive mid and better parent heterosis under control and water stress conditions were 75.27% and 72.14% (control) and 95.53% and 82.78% (water stress) as noticed in highly vigorous F1 combination Hyola-401 × Rohi Sarson (Fig. 2). Nevertheless, under non stress conditions, the minimum but positive mid parent (44.16%) and better parent heterosis (25.87%) observed in Con-I × Rainbow, respectively.

Silique length
For silique length, the positive heterotic results over mid and better parent were noticed in all 15 F1 hybrids under control and water stress conditions (Fig. 3). Positive mid parent heterosis was ranged from 41.52% (Hyola-401 × Rainbow) to 81.14% (Rohi Sarson × Punjab Sarson) under normal water condition, while it was ranged between 42.07% (Hyola-401 × Rainbow) and 84.76% (Punjab Sarson × NARC Sarson) under water stress condition. Positive better parent heterosis was ranged from 38.31% (Hyola-401 × Rainbow) to 80.88% (Rohi Sarson × Punjab Sarson) under normal water condition, while it was ranged between 32.01% (Con-I × Rainbow) and 83.80% (Punjab Sarson × NARC Sarson) under water stress condition.

Seed yield silique
Positive mid parent heterosis was ranged from 41.98% (Hyola-401 × Rohi Sarson) to 92.71% (Con-I × Hyola-401) under normal water condition, while it was ranged between 48.34% (Hyola-401 × Rohi Sarson) and 91.25% (Con-I × Hyola-401) under water stress condition. Positive better parent heterosis was ranged from 33.73% (Con-I × Rohi Sarson) to 70.65% (Con-I × Hyola-401) under normal water condition, while it was ranged between 41.20% (Hyola-401 × Punjab Sarson) and 62.79% (Rohi Sarson × NARC Sarson) under water stress condition (Fig. 4).

Seed yield plant
Positive heterotic effects were seen against mid and better parents in all 15 F1 crosses for seed yield plant under normal and water stress conditions (Fig. 5). The top rank cross combinations over mid parents under control were Hyola-401 × Punjab Sarson (97.11%), Rohi Sarson × Punjab Sarson (92.83%), Con-I × Punjab Sarson (90.28%) and Con-I × Rohi Sarson (88.91%), Hyola-401 × Punjab Sarson (64.72%), whereas under water stress conditions, the highest values of 144.05, 130.13, 126.64 and 121.03% were noted in Rohi Sarson × Punjab Sarson, Punjab Sarson × NARC Sarson, Rohi Sarson × NARC Sarson and Hyola-401 × Punjab Sarson, respectively. Regarding heterosis over better parent under normal water conditions, the top
four hybrids were Rohi Sarson × Punjab Sarson (85.97%), Hyola-401 × Punjab Sarson (84.56%), Hyola-401 × NARC Sarson (80.57%) and Con-I × Rohi Sarson (80.03%); however, under water stress conditions, the highest heterotic effects over high parent were observed in Rohi Sarson × Punjab Sarson (138.98%), Rohi Sarson × NARC Sarson (113.01%), Punjab Sarson × NARC Sarson (112.08%) and Hyola-401 × Rohi Sarson (111.17%).

**Seed index**
The heterotic effects were positive in all 15 F$_1$ crosses for seed index under both the growing conditions (Fig. 6). The maximum mid parent heterosis under control was noted in Rohi Sarson × Punjab Sarson (132.72%), followed Hyola-401 × Punjab Sarson (128.98%), Rainbow × Punjab Sarson (124.82%) and Hyola-401 × NARC Sarson (123.47%), whereas under water stress conditions, the highest values of 104.15, 96.22, 99.29 and 107.57% were noted in same crosses, such as Rohi Sarson × Punjab Sarson, Hyola-401 × Punjab Sarson, Rainbow × Punjab Sarson and Hyola-401 × NARC Sarson, respectively. About heterosis over better parent under normal water conditions, the top four hybrids were Rohi Sarson × Punjab Sarson (132.07%), followed by Hyola-401 × Punjab Sarson (128.44%), Rainbow × Punjab Sarson (123.44%) and Hyola-401 × NARC Sarson (122.51%); however, under water stress conditions, the highest heterotic effects over high parent were observed in Rohi Sarson × Punjab Sarson (106.07%), Hyola-401 × Punjab Sarson (103.66%), Rainbow × Punjab Sarson (97.78%) and Hyola-401 × NARC Sarson (94.87%).

**Chlorophyll (SPAD)**
For chlorophyll SPAD, under non stress and water stress conditions, all 15 F$_1$ crosses demonstrated positive and outstanding results over mid and better parent (Fig. 7). Under non stress and water stress conditions, the hybrids Con-I × Rohi Sarson and Con-I × Punjab Sarson witnessed maximum positive mid parent heterotic effects of 22.15% and 34.65%, respectively. On the other hand, Hyola-401 × Rohi Sarson showed the minimum heterosis over mid parent (12.15% non-stress and 14.45% water stress). Nevertheless, the F$_1$ cross Rohi Sarson × Punjab Sarson disclosed the highest positive results over better parent under non stress (17.41%) and water stress conditions (22.55%).

**Relative water content**
Likewise, other majority characters, the relative water content also showed positive heterotic effects in all F$_1$ hybrids under normal and water deficit conditions (Fig. 8). The highest (21.24%) and lowest (13.86%) positive mid parent heterosis were revealed by Con-I × Punjab Sarson and Rohi Sarson × NARC Sarson, respectively, while the maximum and minimum positive better parent heterosis were 17.31% (Con-I × Rainbow) and 8.64% (Hyola-401 × NARC), respectively. Under water deficit conditions, the F$_1$ cross Con-I × Punjab Sarson disclosed the maximum positive mid (26.64%) and better parent (20.47%) heterosis.

**Oil content**
The positive heterotic results were shown by all 15 F$_1$ hybrids under control and water stress conditions for oil content (Fig. 9). Under control (11.46%) and water stress conditions (13.06%), the highest positive heterotic results over mid parent were observed in same F$_1$ hybrid Con-I × Hyola-401, while for better parent, the maximum positive heterosis observed in Rainbow × Punjab Sarson (10.97% normal and 12.73% water stress). Nevertheless, the minimum positive mid (6.54%) and better parent (6.42%) heterosis as revealed by same F$_1$ cross Rohi Sarson × NARC Sarson under normal conditions, however, under water stress condition, the combination Rohi Sarson × NARC Sarson (7.59%) and Hyola-
401 × NARC Sarson (6.60%) depicted the lowest positive heterosis over mid and better parent, respectively. **Protein content** The exceptional positive heterotic results over mid and better parent found in a group of 15 F₁ combinations under control and water stress conditions (Fig. 10). The maximum positive heterotic effects over mid parent under control (22.39%) and water stress conditions (24.02%) were expressed by Hyola-401 × Rainbow. However, the hybrid Con-I × Punjab Sarson gave the minimum heterotic effects of 7.87% (normal) and 9.58% (water stress) over mid parent. Nonetheless under normal and water deficit conditions, the highest positive better parent heterosis were calculated in Rohi Sarson × Punjab Sarson (8.59%) and Hyola-401 × Punjab Sarson (13.87%), respectively.

![Figure 1. Heterotic effects of F₁ hybrids of rapeseed under control and water stress conditions for plant height](image-url)
Figure 2. Heterotic effects of F1 hybrids of rapeseed under control and water stress conditions for branches plant\(^{-1}\).

Figure 3. Heterotic effects of F1 hybrids of rapeseed under control and water stress conditions for silique length.
Figure 4. Heterotic effects of F₁ hybrids of rapeseed under control and water stress conditions for seed yield silique⁻¹

Figure 5. Heterotic effects of F₁ hybrids of rapeseed under control and water stress conditions for seed yield plant⁻¹
Figure 6. Heterotic effects of F₁ hybrids of rapeseed under control and water stress conditions for seed index

Figure 7. Heterotic effects of F₁ hybrids of rapeseed under control and water stress conditions for chlorophyll SPAD
Figure 8. Heterotic effects of F1 hybrids of rapeseed under control and water stress conditions for relative water content

Figure 9. Heterotic effects of F1 hybrids of rapeseed under control and water stress conditions for oil content
Figure 10. Heterotic effects of F₁ hybrids of rapeseed under control and water stress conditions for protein content

Discussion
The use of heterosis has become an important strategy for increasing crop productivity and breeders must recognize parental lines that are superior to each other through hybridization. Current study showed that there is exceptionally high heterosis of economically important characters including seed yield and oil content is existed in rapeseed parental lines. Several breeders have widely exploited parental genetic potential of various field crops and revealed a great amount of heterotic effects, such as in cotton [17, 18], wheat [19, 20], rice [21, 22], sunflower [23, 24], mustard [25, 26]. In general, positive heterosis is desirable for most the characters, however, Plant height in different crops has a crucial agronomic feature. It affects crop production, oil content and resistance to lodging [27] Medium to tall plant height is considered desirable in rapeseed therefore, F₁ hybrids having medium effects shall be an asset for future breeding programs. High heterosis over better parent under water stress conditions for plant height was reported in crosses Con-I × Hyola-401, Con-I × Rohi Sarson, Hyola-401 × Rohi Srsason, Rainbow × NARC Sarson, Rohi Sarson × NARC Sarson and Punjab Sarson × NARC Sarson (Fig. 1). Past studied reported significant positive mid- and better parent heterosis for plant height in F₁ populations of Brassica napus genotypes [28].

Regarding the characters branches plant⁻¹, silique length, seed yield silique⁻¹ and seed index directly contribute to seed yield showed that none of the hybrid depicted negative heterosis either over mid or better parent under normal and water stress conditions (Fig. 2-6). However, the maximum positive heterosis over better parent under control and water stress conditions were displayed by Hyola-401 × Rainbow, Hyola-401 × Rohi Srason, Hyola-401 × Punjab Sarson, Hyola-401 × NARC Sarson, Rainbow × Rohi Sarson and Rohi Sarson × Punjab Sarson for branches plant⁻¹
The extent of heterosis over better in this study was quite higher than those reported by [29], however, [28] observed higher heterosis than this study in some set of crosses for branches plant$^{-1}$ as previously recorded by other breeders of oilseeds [30, 31, 32, 29]. The top rank heterotic effects for the characters silique length and seed yield silique$^{-1}$, the maximum and positive heterosis over high performance parent was calculated in Rainbow × Punjab Sarson, Rainbow × NARC Sarson, Rohi Sarson × Punjab Sarson, Rohi Sarson × NARC Sarson and Punjab Sarson × NARC Sarson under both growing conditions (Fig. 3 & 4). Under normal and water stress conditions for seed index, the top five crosses with the highest heterosis over better parent were Hyola-401 × Punjab Sarson, Hyola-401 × NARC Sarson, Rainbow × Punjab Sarson, Rohi Sarson × Punjab Sarson and Punjab Sarson × NARC Sarson (Fig. 6) the obtained results showed higher heterotic effects than those revealed by [33, 34, 35, 36]. Current study also identified numerous F$_1$ hybrids with positive heterotic effects for seed index that were in conformity with the earlier findings of [32, 29, 35, 28] who reported significant positive mid and better parent heterosis for seed index in Brassica napus. For launching stress breeding program, it is very important to examine physiological parameters, therefore, the current study involved two key stress breeding characters, such as relative water content and chlorophyll SPAD. For both above mentioned traits, the top three F$_1$ hybrids over best parent under water stress were Con-I × Punjab Sarson, Rainbow × Punjab Sarson and Rohi Sarson × Punjab Sarson (Fig. 7 & 8). Thus, these three hybrids may be utilized to develop vigorous hybrids of rapeseed genotypes.

Regarding heterotic effects for seed yield plant$^{-1}$, a substantial amount of heterosis over better parent (138.98%) was noticed in Rohi Sarson × Punjab Sarson under water stress conditions (Fig. 5). Various researchers reported different extent of better parent heterosis, however, that limit of heterosis was lower as compare with current results, like [34] observed 41.15% best parent heterosis, [32] found 35.9% high parent heterosis, [29] noticed 125.71% heterobeltiosis and [28] witnessed 132.54% better parent heterosis for yield plant$^{-1}$. More importantly, all 15 F$_1$ hybrids disclosed positive mid and better parent heterosis either under normal or water stress conditions, representing the genetic potential of breeding materials used in the study. One of the most important targets in rapeseed production is the high oil content and good heterotic effects are considered useful for selecting superior oil production hybrids (Fig. 9). In this study, heterotic effects for oil content were higher than recorded [32]. However, on the other hand, various oilseed breeders, such as [37, 38, 39] reported negative or no heterosis for oil content in oil seed Brassica.

**Conclusion**

Considering the heterotic effects, the crosses Rohi Sarson × Punjab Sarson, Hyola-401 × Punjab Sarson and Hyola-401 × NARC, showed the highest heterosis over better parent, with respect to seed yield plant$^{-1}$ and oil content, under normal and water stress conditions, respectively. These findings of the potential hybrids show their worth for heterosis breeding and these materials can be used in future rapeseed breeding programs for the evaluations of potential verities.

**Authors’ contributions**

Conceived and designed the experiments: SH Rao & S Bano, Performed the experiments: SH Rao, LA Bhutto & R Akram, Analyzed the data: SH Rao, SA Channa & M Arsal, contributed reagents/ materials/ analysis tools: SH Rao, H Khan & Asadullah, Wrote the paper: SH Rao & AW Baloch

**References**