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EVALUATION OF RAPESEED (*BRASSICA NAPUS* L.) GENOTYPES FOR WATER STRESS CONDITION



Sajid Hussain Rao¹, Abdul Wahid Baloch^{1*}, Siraj Ahmad Channa¹, Liaquat Ali Bhutto²,
Saima Bano¹, Naila Gandahi¹

¹Department of Plant Breeding & Genetics, Sindh Agriculture University, Tandojam, Pakistan

²Agriculture Research Center, Tandojam, Pakistan

*Corresponding Author: Abdul Wahid Baloch. E-mail: awbaloch@sau.edu.pk

Abstract

Water shortage is a key abiotic issue that restricts agricultural yield in several parts of Pakistan, as well as globally. Despite many attempts made over the last several years, rapeseed crop productivity has remained unchanged, even though there is a lot of genetic diversity in rapeseed genotypes for seed production and other economic characteristics. In this study, a set of 20 rapeseed cultivars were tested for resistance to water stress in natural field using a randomized complete block design (RCBD) in a factorial setting with four replications for six different traits, such as, seed yield plant⁻¹ (g), seed index (1000-seed weight, g), relative water content (%), SPAD chlorophyll, oil content (%) and protein content (%), while three water systems (Treatments = T) were applied for this investigation. T₁ was considered as control with four irrigations; T₂ was considered as water stress at maturity with three irrigations and T₃ was regarded as water stress at silique formation with two irrigations. Mean squares of different sources (genotypes, water treatments, and their interactions) were significant for all studied characters, urging the importance of these genetic resources for future breeding. Under water stress conditions, a set of six genotypes of rapeseed, such as, NARC Sarson, CON-1, Punjab Sarson, Rainbow, Rohi Sarson and Hyola-I showed high mean values for above mentioned traits hence were tagged as potential rapeseed genetic stock for water stress breeding. This suggests that these rapeseed genotypes might give valuable genetic recombination for water stress situations, and hence could be used in future breeding efforts.

Keywords: Drought, Canola, Genetic variation, Seed yield, Physiological parameters

INTRODUCTION

Rapeseed (*Brassica napus* L.) is an important oilseed crop, which is largely grown for oil, condiments, vegetables, or fodder (1). During 2020-21, the availability of total edible oil in Pakistan was 3.29 million tonnes, while only 0.374 million tonnes *i.e.*, 13% of total availability was locally produced and 87% was imported by spending 574.19 billion rupees (2). For breeding rapeseed crop, a series of experiments have been conducted during previous years; however, productivity has still remained almost stagnant. This may be due to a lack of high yielding genotypes with unstable performance over the environment, lack of genetic variability for some important yield components. It possesses enormous genetic variability for seed and drought tolerance characteristics, which further leads to genetic diversity (3). To increase yield, the study of genetic parameters provides the basis for launching successful breeding program. Genetic variability is the basic requirement for making progress and improving crops, which further leads to progress in crop breeding. Thus, there are good chances to improve this crop by evaluating and utilizing genetic variation (4). Water stress is a main abiotic factor, which limits the productivity of major crops in numerous areas of the world (5). In water deficit condition, tolerance genotypes reveal greater capability



of adaptation for agronomical performance, which is a good indicator for such breeding materials to be used in stress breeding programs. Water stress causes great damage to agricultural crops by showing adverse effects on their development and growth, leading to low production (6). Keeping the above facts, a set of 20 rapeseed genotypes were assessed for their genetic variations against water stress condition with respect to identify tolerant rapeseed genotypes for poor resource farmers with minimum loss in seed yield.

MATERIALS AND METHODS

BREEDING MATERIALS, EXPERIMENTAL DESIGNS AND WATER TREATMENTS

The purpose of this research was to screen out rapeseed genotypes for water stress conditions under field conditions. The experiment was conducted at experimental field, Sindh Agriculture University, Tandojam during the rabi season 2017-18. A set of 20 rapeseed cultivars (Punjab sarson, Rohi sarson, NARC sarson, PARC canola, Faisalabad canola, Hyola-401, Rainbow, Pakola, Con-I, Con-II, Con-III, Waster, Dunkled, Abasion-95, Hyola-42, Hb0-555, Mun-I, Kungola, Hbo-63 and Kn-277) were tested for resistance to water stress in a natural field using RCBD in a factorial setting with four replications for six different traits. Moreover, three water systems (Treatments = T) were applied for this investigation. T₁ was considered as control/normal with four irrigations, T₂ was considered as water stress at maturity with three irrigations and T₃ was regarded as water stress at silique formation with two irrigations.

CULTURAL PRACTICES

The plant to plant and row to row distances were kept at 10 and 30 cm, respectively. The recommended fertilizer dose such as N:P:K was applied at the rate of 40:24:20 kg acre⁻¹. This dose of fertilizer was applied in split doses; the half of the dose was applied before sowing while half of the dose was applied at first irrigation. For weeding, three hoeing at the interval of two weeks were done with respect to control weeds. For controlling aphid, the Confider pesticide was used at the rate of 250mL acre⁻¹, when economic threshold was observed.

TRAITS INVESTIGATED

AGRONOMICAL AND PHYSIOLOGICAL CHARACTERIZATION

The obtained seeds from each plant were weighed in grams and termed it as seed yield plant⁻¹. 1000 seed weighed in grams as seed index was recorded after hand threshing of siliques. Whereas SPAD (Soil Plant Analysis Development) chlorophyll meter (Model No: SPAD-502 Plus) was used for obtaining the data of SPAD chlorophyll. (7) technique was used to determine the relative water content (RWC%). For this RWC%, the leaf sample was weighed fresh (FW), wilted in distilled water for 4 hours, and turgid weight (TW) was determined, after which the leaves were dried for 24 hours at 72 °C for gaining dry weight (DW). Following formula was used to calculate relative water content.

$$\text{RWC}\% = [(\text{FW}-\text{DW}) / (\text{TW}-\text{DW})] \times 100$$

EXTRACTION OF OIL AND PROTEIN CONTENT (%)

By collecting 5 to 10 grams seed samples and drying them in the oven, the oil content was determined using the Soxhlet extract technique. After that, took 2 to 3 grams of dried seed samples and made the individual packages. After that, the dried packages were placed in a Soxhlet device. The petroleum ether was then placed in a chemical flask and put into the Soxhlet device. When the extract became clear, the samples were removed from the Soxhlet apparatus and dried in fresh air before being placed in the oven. Finally, use the method below to determine the reading (8).

$$\text{Initial reading} - \text{final reading} / \text{initial reading} \times 100$$

Protein content was assessed using the American Oil Chemists' Society (AOCS) official technique, also known as the combustion method for estimating crude protein, which was reapproved in 1997. Using this method, a sample of grains was crushed and cooked at a very high temperature (900 °C) in the presence of oxygen. Carbon dioxide, water, and nitrogen were released as a result of the combustion. The 6.25 factor

was used to translate the measured nitrogen content into crude protein content. The results were expressed as a percentage, with N6.25 signifying nitrogen and 6.25 denoting an oilseed component.

PRECIPITATION AND TEMPERATURE DATA

According to Agro-Meteorological Data Regional Agromet Centre, Tandojam. There was no rain fall during the months of experimentation *i.e.*, October 2017 to March 2018. The temperature was also recorded of the experimental site (Table I).

Table I. Total rain and temperature recorded in during experiment

Months	Total rain (mm)		Temperature			
	Total	Means	Min. °C		Max. °C	
			Total	mean	Total	Mean
October-2017	0.0	0.0	603.0	19.5	1189.5	38.4
November-2017	0.0	0.0	376.5	12.6	936.5	31.2
December-2017	0.0	0.0	239.5	8.0	768.5	25.6
January-2018	0.0	0.0	248.0	8.0	821.0	26.5
February-2018	0.0	0.0	332.0	11.9	807.0	28.8
March-2018	0.0	0.0	488.0	15.7	1123.0	36.2

STATISTICAL ANALYSIS

Variance analysis was performed in accordance with (9), while Least Significant Differences (LSD) test was used to determine the superiority of the treatment means.

RESULTS AND DISCUSSION

Analysis of variances: The significant difference ($P \leq 0.05$) mean squares for genotypes, treatments and their interaction was observed for all the parameters, such as relative water content, SPAD chlorophyll, seed yield plant⁻¹, seed index, oil content and protein content (Table 2), showing that lot of genetic potential is existed in these genotypes of rapeseed that may be used in a variety of breeding efforts to enhance the genetics of this crop. According to (10), water deficiency stress considerably impacted certain qualitative and quantitative characteristics of rapeseed genotypes. In another study, 15 canola genotypes with drought resistance based on yield and associated characteristics were assessed. The mean squares revealed substantial yield differences between the rapeseed germplasm in both normal and water stress circumstances (11). Each character is detailed in the following paragraphs.

Table II. Mean squares of various traits of rapeseed genotypes

Source of variances	Degree of Freedom	SPAD chlorophyll	Relative water content	Seed yield plant ⁻¹	Seed index	Oil content	Protein content
Replication	3	14.19	0.01494	22.415	0.22721	0.005	0.80
Genotypes	19	281.39**	0.01100*	6.309**	0.22872*	26.215**	53.455**
Treatments	2	1113.95**	0.13778**	132.348**	2.42100**	221.584**	88.939**
G x T interaction	38	22.32**	0.00328 ^{ns}	1.209 ^{ns}	0.02371 ^{ns}	3.994**	0.6226**
Error	177	8.11	0.00618	2.986	0.11763	0.062	0.0253

** and * indicates significant level at 1% and 5% of probability level, whereas ns indicates non-significant

Table III. Mean and range of various traits in control and water stress conditions of rapeseed genotypes

Averages and range		SPAD chlorophyll	Relative water content (%)	Seed yield plant ⁻¹ (g)	Seed index (g)	Oil content (%)	Protein content (%)
Average	Control	47.49	76.87	9.79	3.63	39.32	26.48
	Water stress at maturity	42.17	73.27	8.53	3.41	37.59	25.52
	Water stress at silique	40.30	68.59	7.22	3.29	35.99	24.50
Range	Control	43.12-53.37	70.00-85.00	7.87-12.40	3.36-3.94	36.46-41.51	20.66-29.52
	Water stress at maturity	33.96-49.69	67.00-80.00	6.92-9.84	3.19-3.68	32.71-40.11	19.80-28.35
	Water stress at silique	31.03-47.18	60.00-77.00	5.87-8.44	2.99-3.57	30.92-39.75	19.91-27.26

CHLOROPHYLL (SPAD)

The leaf chlorophyll plays a role in determining the pace of photosynthesis and the amount of dry matter produced (12). Plant growth can be limited under water stress by varying chlorophyll concentration and respiration (13). The current study's findings clearly showed that a high amount of water shortage has negative impacts on chlorophyll. Nonetheless, there were substantial differences in drought tolerance among the rapeseed cultivars studied. Although the genetic differences in responsiveness of the tested genotypes may be largely attributed to the genetic composition of agricultural plants, whereas number of genotypes also performed exceptionally well for chlorophyll under water stress circumstances (Fig. 1). Different genotypes performed admirably for chlorophyll under both water stress (maturity and silique stages) conditions. Under control, maturity and silique stages, the chlorophyll levels ranged from 43.12 to 53.37, 33.96 to 49.69, and 31.03 to 47.18, respectively. The highest chlorophyll values of 53.37 (NARC Sarson) under control, 49.69 (Kungola) at maturity water stress and 47.18 (Pakola) at silique water stress, while the lowest chlorophyll values were found in Con-II, Abasin-95, and Hyola-42 under control, maturity and silique water stresses with the values of 43.12, 33.96, and 31.03, respectively. Overall, the genotypes NARC Sarson, Kungola, and Pakola performed well in water stress conditions. In compared to drought-tolerant rapeseed genotypes, drought-sensitive rapeseed genotypes showed a higher decrease in chlorophyll. The trend was not different from earlier research, in which water deficiency had a negative impact and there was a large drop in chlorophyll. The genotypes of rapeseed showed varied responses to water stress, suggesting that they had different genetic potential for drought resistance. According to (14), water stress in agricultural plants is predicted to cause several physiological problems, such as a decrease in chlorophyll content, photosynthesis, and respiration.

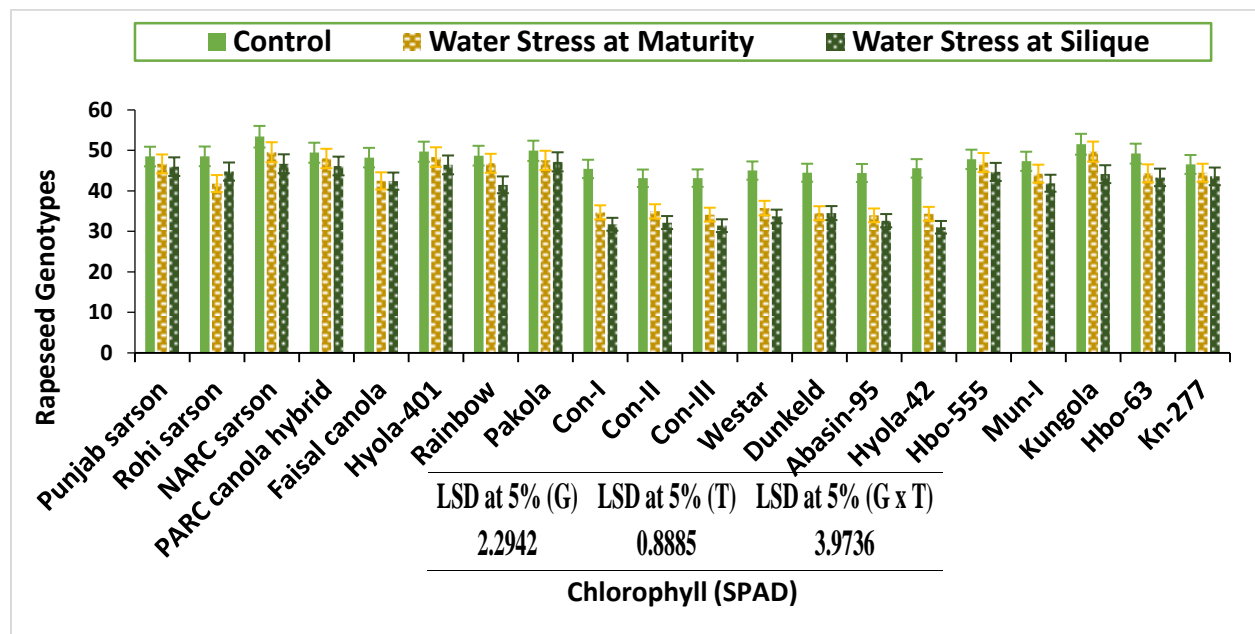


Fig. 1. Mean values of chlorophyll (SPAD) in control, water stress at maturity and silique formation

RELATIVE WATER CONTENT (RWC %)

RWC is an important metric trait for determining the condition of water in plants (15). RWC represents a plant's reaction to diverse environmental circumstances; nevertheless, RWC has been found to be a more powerful and stable characteristic than leaf water potential (16). Water stress has a major impact on the growth, development, and yield of agriculturally essential crops; therefore, genotype screening for rapeseed genotypes is critical (6). The genotypes like Pakola (85%), NARC Sarson (80%), and Hyola-401 (77%) provided the greatest RWC at three treatments, including control, maturity and silique stage water stresses, respectively (Fig. 2). Con-II had the lowest RWC (70%) under normal water conditions, whereas Abasin-95 (67%) and Hbo-555 (60%) had the lowest RWC during maturity and silique formation water stresses, respectively. The average RWC under control, maturity, and silique phases were found to be 77%,

73%, and 69%, respectively (Table 3). According to (17), water stress reduces the performance of several characteristics such as relative water content, osmotic potential, and potassium content when compared to control. Nonetheless, the genotypes NARC Sarson and Hyola-401 showed promising results in terms of RWC under water-stressed circumstances. The stress imposed by water significantly reduced the relative water content as also reported by various researchers (18, 19).

SEED YIELD PLANT⁻¹ (g)

The maximum seed yield plant⁻¹ (g) under control, stress at maturity stage, and stress at silique stage were generated by Punjab Sarson and Hyola-401 (Fig. 3). On the other hand, Kn-277 had the lowest seed yield plant⁻¹ under control, whereas PARC canola hybrid had the highest seed yield plant⁻¹ at maturity and silique stress phases. Furthermore, under normal conditions, seed yield plant⁻¹ varied from 7.87 to 12.40 g and from 6.92 to 9.84 g during maturity water stress stage and from 5.87 to 8.44 g at silique water stress phase. In terms of averages, the control, maturity, and silique stages yielded 9.79, 8.53, and 7.22 g, respectively (Table 3). The results indicated that genotypes Punjab Sarson and Hyola-401 generated the greatest seed yield plant⁻¹ (g) under three water treatments, suggesting that these genotypes should be prioritized in the selection and hybridization program to maximize seed production. Water stress accelerates flower and fruit shedding, resulting in a decrease in seed production owing to a lack of water during the reproductive period (20, 21). When water was applied once in the spring, (22) reported the greatest drop in seed production (23). Similarly, found that moderate and acute water stress reduced seed production and other agronomic characteristics.

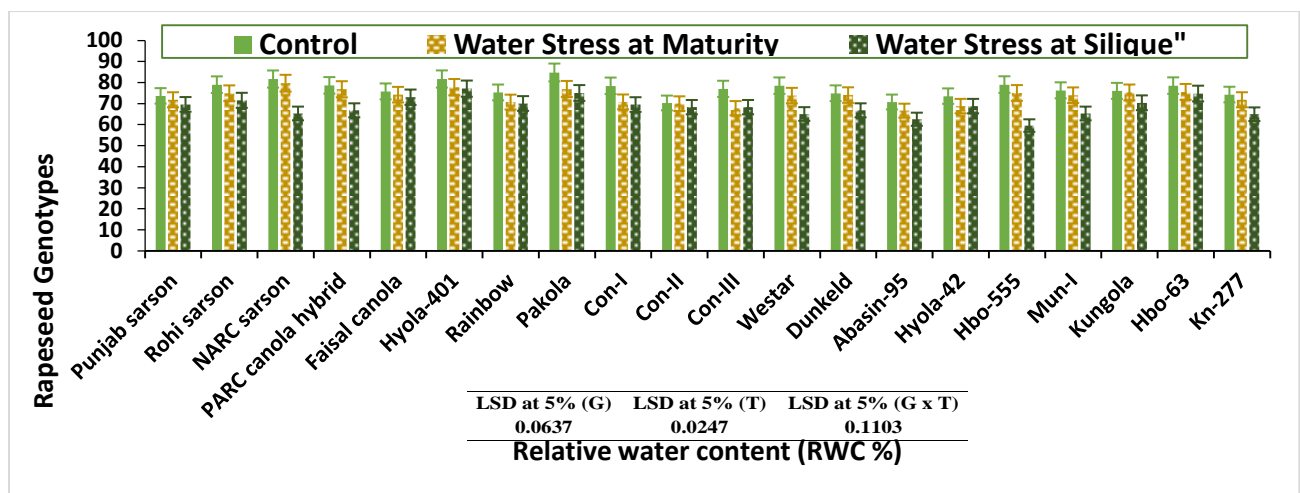


Fig. 2. Mean values of relative water content (RWC %) in control, water stress at maturity and silique formation

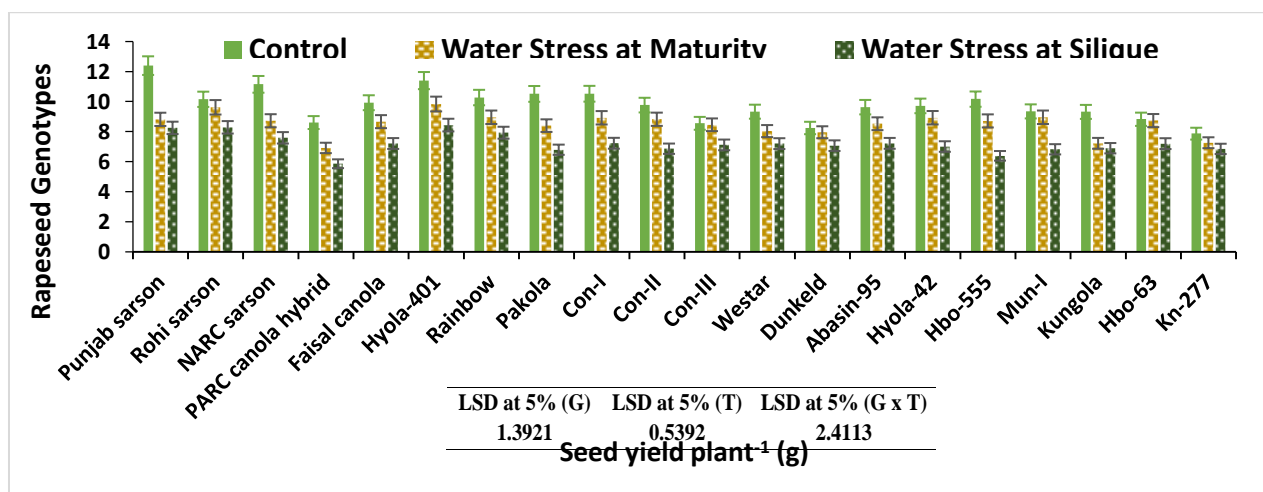


Fig. 3. Mean values of seed yield plant (g) in control, water stress at maturity and silique formation



SEED INDEX (1000-SEED wt, g)

In three water treatments (control, stress at maturity, and stress at silique formation), the genotypes Rohi Sarson, Pakola, and Hbo-555 generated the heaviest seeds in terms of seed index such as 3.94 g, 3.68 g and 3.57 g, respectively (Fig. 4). On the other hand, the genotypes Kungola (3.36 g) under control, Hbo-63 (3.19 g) at maturity water stress stage, and Faisal canola (2.99 g) at silique water stress stage had the lowest seed index. Furthermore, in different growth circumstances, larger genetic differences in seed index were observed. The average of 3.63, 3.41 and 3.29 g were recorded for seed index in all above three conditions (Table III). Malekei and Sinaki (24) also reported that irrigation intervals had an effect on seed weight. Water stress is also a risk factor for rapeseed genotypes, resulting in a significant drop in seed index (25). Similarly, a significant drop in 1000-seed weight in rapeseed cultivars owing to water stress (26). Furthermore, Shirani et al. (10) viewed that water shortage affects both quantitative and qualitative characteristics in canola cultivars. According to the findings, the genotypes Rohi Sarson, Pakola, and Hbo-555 had the greatest seed index under normal water, silique, and maturity water stresses, as well as a higher average seed index under water stress. Nonetheless, these rapeseed genotype genetic stocks will be preferred breeding material in future rapeseed breeding projects.

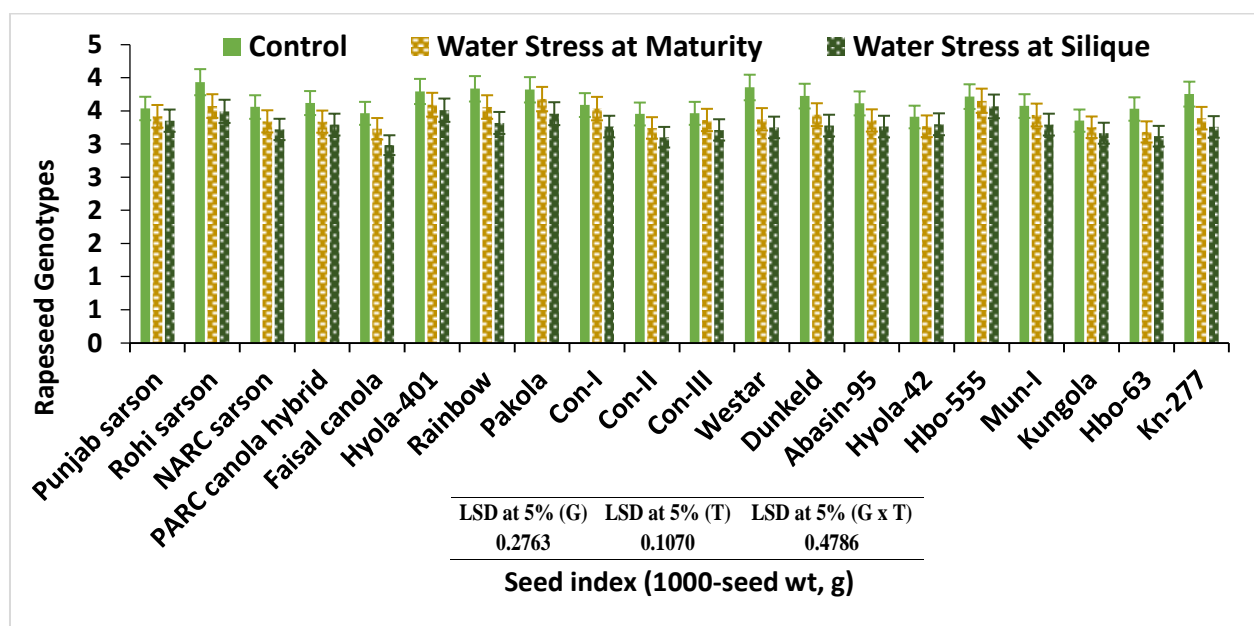


Fig. 4. Average values of seed index (g) in control, water stress at maturity and silique formation

OIL CONTENT (%)

The major qualitative features of rapeseed plants are oil content, which are substantially impacted by water deficiency (27). With respect to oil content under control, maturity, and silique phases, genotype oil content varied from 36.46 to 41.51%, 32.71 to 40.11%, and 30.92 to 39.75%, respectively (Fig. 5) (Table III). Under full irrigation circumstances, genotype Rainbow produced the highest oil content (41.51%), whereas Hyola-401 provided the highest oil content (40.11% and 39.75%, respectively) at maturity and silique water stresses. Dunkeld generated the lowest oil content of 36.46% and 32.71% in the control and maturity phases, respectively, while Kungola (30.92%) provided the lowest oil content at the silique stage. The findings of oil content revealed that genotypes rainbow and Hyola-401 generated the greatest oil content; however, these rapeseed genotypes would be good breeding material for oil content. Irrigation intervals had a significant influence on oil %, according to (24). The low seed oil content of rapeseed plants was discovered as a result of a water shortage during the blooming period (28). According to (26), a reduction in watering intervals reduces seed oil production.

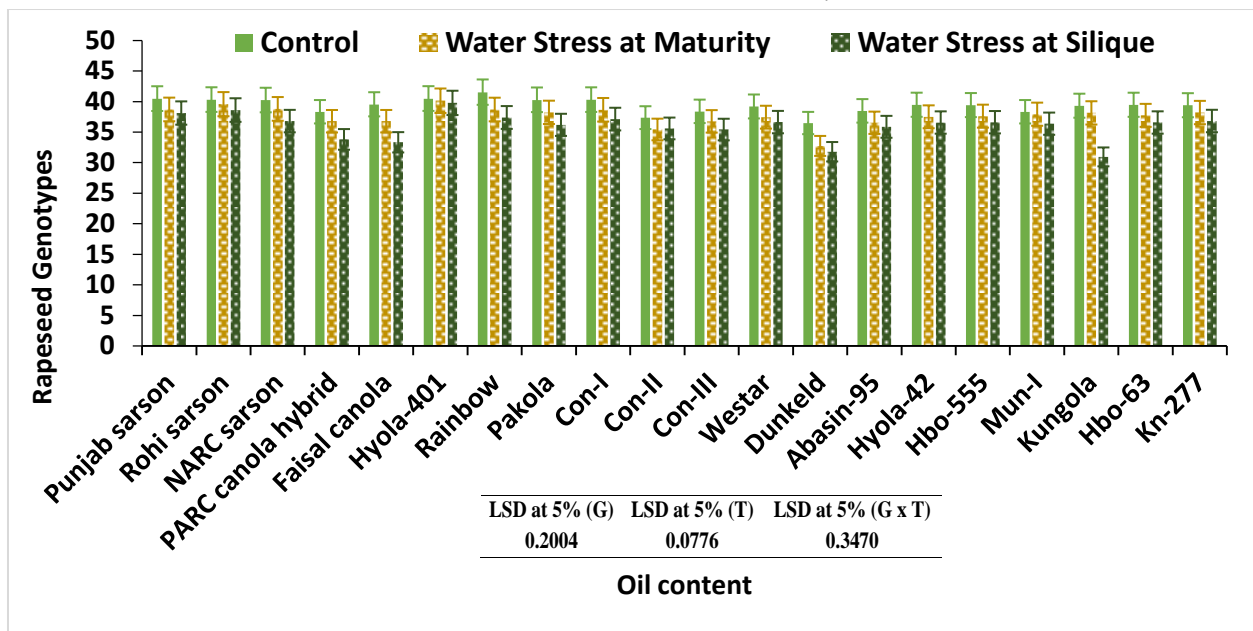


Fig. 5. Average values of oil content (%) in control, water stress at maturity and silique formation

PROTEIN CONTENT (%)

Considering the protein content in normal conditions, the protein content (%) ranged from 20.66 to 29.52%; in water stress maturity, it ranged from 19.80 to 28.35%; and in water stress at silique, it ranged from 19.91 to 27.26% (Fig. 6). Under regular water conditions, maturity and silique phases, the protein content was averaged as 26.48, 25.52, and 24.50%, respectively (Table III). Rainbow canola hybrids had the highest protein content under controlled conditions (29.52%). The greatest protein content was found in Faisal canola (28.35%) at silique water stress and followed by PARC canola hybrid (28.15%) and Rainbow (27.64%). Rainbow (27.26%) genotypes generated the highest protein content at the silique water stage (29) found that under drought stress, total protein synthesis dropped by a substantial amount.

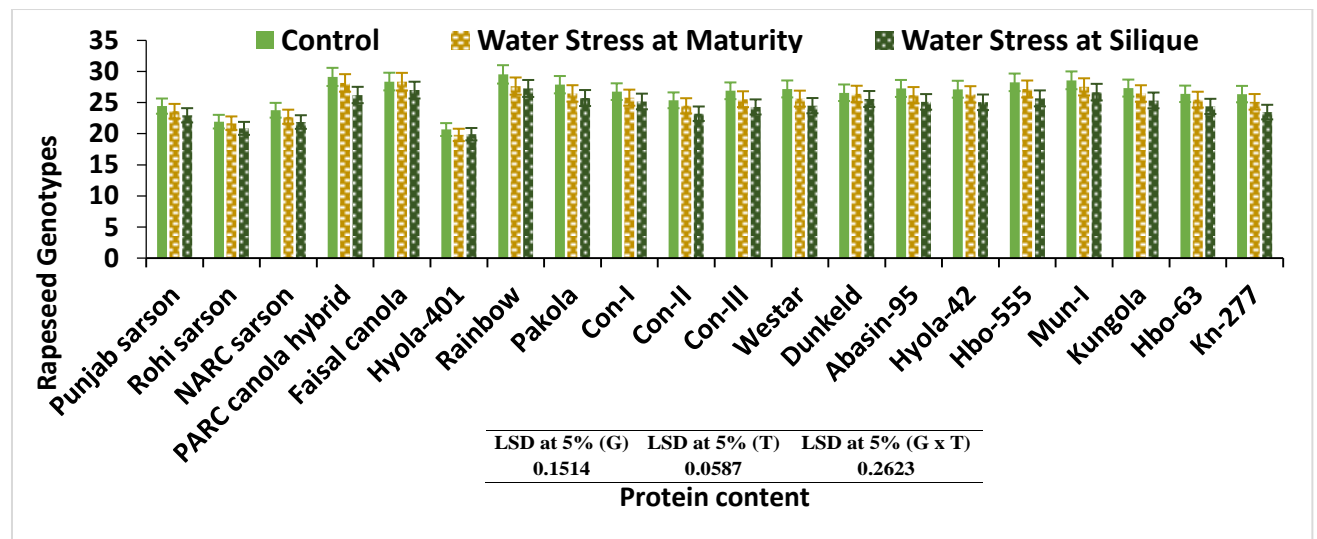


Fig. 6. Average values of protein content (%) in control, water stress at maturity and silique formation

CONCLUSION

It is concluded that based on average performance under three water systems, the set of six genotypes of rapeseed such as NARC Sarson, CON-1, Punjab Sarson, Rainbow, Rohi Sarson and Hyola-I were identified as potential rapeseed genetic stock for water stress breeding and poor resource farmers may grow these genotypes under limited water with minimum loss in seed yield.

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Conflicts of interest:

The authors declare no conflict of interest.

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