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Contribution of drought on variability in crop yields and physiochemical responses of selected Rapeseed/mustard genotypes

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Abstract

To identify the drought-tolerant Rapeseed-mustard genotype based on physiochemical responses an experiment was carried out from November 2017 to February 2018. The experiment was designed in Randomized Complete Block (RCB) with three replications including the treatments of factor A: irrigation regimes *viz.* T_1 = irrigation (control) and T_2 = drought (no irrigation) and factor B: five genotypes/varieties *viz.* V_1 = BC-9909, V_2 = BJDH-05, V_3 = Nap-0544, V_4 = BARI Sharisha-11, and V_5 = BARI Sharisha-16. Irrespective of genotypes/varieties, drought stress greatly fluctuated the all physiochemical, yield contributing and yield parameters. Based on physicochemical parameters like accumulation of chlorophyll, enzymatic and non-enzymatic antioxidant; Nap-0544 was found to be a drought-tolerant genotype due to its greater and lower accumulation of enzymatic and non-enzymatic antioxidant respectively (greater CAT, POD, APX, proline and lower MDA) under drought stress. This genotype may be further explored to characterize its genes and mechanisms against drought stress for increased Rapeseed-mustard production and way of developing the drought-tolerant varieties.

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Introduction

An extreme water deficit condition is the most common ecological threats for crop production that finally restrict to ensure food security for any nations. Sustainable crop production has been a challenging issue for many developing countries due to greater vulnerabilities and incomplete to mitigate the adverse effect of climate change (Ali *et al.* 2017). The climate change issue like the sensitivity of drought varies with the crop species along with their respective growth stage (Qiang *et al.* 2016). Morphological and physiological variations under drought stress are the reflection of plant genetic diversity in drought tolerance. The genotypes with high adaptability to drought stress can be a candidate as genetic resources to improve against drought tolerance varieties. Drought impacts on changes stomata conductance, osmolyte accumulation, growth, yield, photosynthetic activity, pigment content, water relations in plant body (Praba *et al.* 2009). Earlier researches revealed that subjecting to water stress at various growth stages grain yield was reduced 40% in maize (Çakir 2004), 9 to 45% in Soybean (Eck *et al.* 1987), 27.5% in Wheat, 25.4% in Rice (Zhang *et al.* 2018), 6 to 54% in Lentil (Oweis *et al.* 2004). Therefore, the survival of the plants of any stress depends on the capacity of the plants to quickly adapt to changing energy equations, the intensity of stress and the growing environment (Miller *et al.* 2010). Plants generally adopted different mechanisms such as morphological, physiological and biochemical to increase tolerance against stress. The detrimental effects of stress lead to oxidative stress which causes the over-accumulation of ROS in the plant body. Primarily plants deal with oxidative stress via an endogenous defensive mechanism consisting of a different enzymatic and non-enzymatic antioxidant such as peroxidase (POD), (SOD) superoxide dismutase, (CAT) catalase, (APX) ascorbate peroxidase and ascorbate (AsA) and glutathione (GSH) as well as tocopherol, carotenoids and phenolic compounds, etc. However, the generation of ROS demolishes the stability in the plant body and causes cellular damage, leading to programmed cell death (PCD) as well as decreasing plant productivity during stress conditions (Raja *et al.* 2017). Programmed cell death is an active, genetically controlled process in which cells are selectively eliminated in a highly coordinated, multi-step fashion through the involvement of specific proteases and nucleases. So, Assessment and identify drought-tolerant genotypes are critical to all studies concerning drought tolerance (Cattivelli *et al.* 2008). With this background, rapeseed-mustard is an important oil-producing crop of Bangladesh both based on its total cultivated area and production, respectively. The present yield of rapeseed-mustard (0.95 tha^{-1}) (Biswas *et al.* 2019) is very low as compared to other oilseeds growing countries in the world. The main reasons for lower yield may be a lack of resistant genotypes/varieties against different abiotic stress, good quality seeds and inadequate adoption of improved production technologies. Addressing physiological responses earlier researches were well documented by the renowned researcher for identifying the drought-tolerant genotypes/varieties of crops like rice (Pervin *et al.* 2017), Chickpea (Shah *et al.* 2020), Cotton (Penna *et al.* 1998), Whet (Haque *et al.* 2020), Maize (Badr *et al.* 2020). Though the effects of drought stress have been well-documented in many crop species reports addressing physiological responses on oilseed crops were found relatively limited. However, the present study was conducted to identify the drought-tolerant Rapeseed-mustard genotype based on physiological responses.

Materials and methods

To evaluate the yield differences and physiochemical mechanism of Rapeseed-mustard genotypes against drought stress a pot experiment was conducted in the Plant Physiology Division of BARI. The experiment was conducted from November 2017 to February 2018. The experiment was conducted including three Rapeseed-mustard genotypes *viz.* BC-9909, BJDH-05, Nap-0544 and two released varieties *viz.* BARI Sharisha-11 and BARI Sharisha-16.

The test crops were grown under irrigation (control) and drought (no irrigation) conditions. The control plants were maintained with proper irrigation started

at 30 days after sowing (DAS) while the other set of plants were subjected to water stress by withholding irrigation during the whole growing period. The study was laid out in Randomized Complete Block Design with six replications and each pot was considered as one replication. To experiment a total of 60 (26 cm top diameter, 20 cm base diameter and 25 cm in height) pots were arranged with the placement of 30 pots in two replicate blocks in the pothouse. Soil and well-decomposed farmyard manure were mixed properly in a 4:1 volume ratio and kept in each pot containing 12 kg of soil. Fertilizers @ 100-30-80-20-3.0-1.0 kg ha^{-1} of N-P- K-S-Zn-B were applied in the form of urea, triple superphosphate, muriate of potash, sulphur and zinc sulphate and boron, respectively. Provax-200 WP treated seeds were sown on 27 November 2017. Seven to ten healthy and uniform size seeds were sown in each pot as per the variety arrangement. Subsequently, five healthy seedlings in each pot were maintained. Crop growth and physiological parameters were recorded with time. To measure the physiological parameters sampling was done on sunny days within the period from 11.00 am to 1.30 pm when drought stress symptoms were visible in crops.

Physiological parameter

Photosynthetic pigment (Chlorophyll accumulation) of leaves (mg g^{-1} FW^{-1})

The second or third leaf sample from the plant of each plot was collected and weighed out in 0.5 g (fresh weight). Then the sample was treated using 10 ml (V) of 80% acetone for approximately 48 hours until the leaf turned white under dark conditions.

The optical density was measured with UV-1800 spectrophotometer (Shimadzu, Japan) against 80% acetone as blank at 663nm (OD663) and 645 nm (OD645) for chlorophyll a (Chl a) and chlorophyll b (Chl b), respectively. The chlorophyll concentrations (Chl) were determined using the following formula as described by Arnon (1994).

Chl a (mg g^{-1}) = [12.7 (OD 663)-2.69(OD645)] × v/ (1000×W)

Chl b (mg g^{-1}) = [22.9 (OD 645)-4.68(OD663)] × v/ (1000 ×W)

Total Chlorophyll (mg g^{-1}) = [20.2 (OD 645) + 8.02 (OD 663)] × V/ (1000×W).

Bio-chemical analysis

Enzyme extraction and assays

To perform biochemical analysis fully expanded 3rd leaf from the top of the plant of each plot was collected and kept in a laboratory within a zipper bag keeping in an icebox. Using a pre-cooled mortar and pestle, 0.5 g of leaf tissue was homogenized in 8 ml of 50 mM ice-cold Tris-HCl buffer (pH 7.2) containing 1mM $Na_2HPO.12H_2O$ and 1mM $NaH_2PO4.2H_2O$. The homogenates were centrifuged at 10,000×g for 20 minutes. Supernatants were collected after centrifugation and used to determine biochemical compounds such as CAT, POD, APX and MDA. The collected supernatant was stored at 4 °C temperature until use. All biochemical activities were performed by SHIMADZU UV spectrophotometer (UV-1800).

Catalase (CAT, EC: 1:11:1.6): Catalase activity was carried out in a 3-ml reaction volume containing 2.8 ml of 50 mM Tris-HCl buffer (pH 7.2, not containing EDTA), 100 µl of enzyme extract and 100 µl of 300 mM H2O2 from 30% H_2O_2 was taken in a quevette which was placed in measuring chamber of UV spectrophotometer. The activity was determined at 240 nm wavelength, which measures the decrease in absorbance for 30 seconds. The activity was calculated using the extinction coefficient of 39.4 mM cm^{-1} according to Wu *et al.* (2003).

Peroxidase (POD, EC 1.11.1.7): POD activity was carried out in a 3-ml reaction volume containing 2.7 ml of 50 mM Tris-HCl buffer (pH 7.2, not containing EDTA), 100 µl of enzyme extract and 100 µl of 1.5% Guaicol, 100 µl of 300 mM H2O2 from 30% H_2O_2 was taken in a quevette which was kept in measuring chamber of UV spectrophotometer. The activity was determined at 240 nm wavelength, which measures the decrease in absorbance for 1 min. The activity was calculated using the extinction coefficient of 39.4 mM cm^{-1}according to Wu *et al.* (2003).

MDA (Lipid peroxidation) assays: Malondialdihyde (MDA) (lipid peroxidation) was measured as per the protocol of Wu *et al.* (2003). 1.5 ml plant enzyme extract and 2.5 ml reaction solution (5%

Trichloroacetic acid + 0.6% thiobarbituric acid) were mixed in a small tube and gave in a hot water bath at 95 °C for 15 minutes and then gave immediately in an ice bath. Subsequently, the reaction solution was centrifuged @ 4800 rpm for 10 minutes.

The absorbance of the supernatant was recorded at 532 nm. Correction of non-specific turbidity was made by subtracting the absorbance value read at 600 nm. The level of lipid peroxidation was expressed as nmol g^{-1} fresh weight, with a molar extinction coefficient of 0.155 $mMcm^{-1}$.

Ascorbate peroxidase (APX, EC: 1.11.1.11) activity was assayed following the method of Chen *et al.* (2010). The reaction buffer solution contained 50 mM K-phosphate buffer (pH 7.0), 0.5 mM ASC, 0.1 mM H_2O_2, 0.1 mM EDTA, and enzyme extract in a final volume of 0.7 ml. The reaction was started by the addition of H_2O_2, and the activity was measured by observing the decrease in absorbance at 290 nm for 1 min using an extinction coefficient of 2.8 mM^{-1} cm^{-1}.

Proline assessment

Proline was extracted from a sample of 0.5 g fresh leaf material samples in 3% (w/v) aqueous sulphosalycylic acid and estimated using the ninhydrin reagent according to the method of Bates *et al.* (1973).

The absorbance of the fraction with toluene aspired from the liquid phase was read at a wavelength of 520 nm. Proline concentration was determined using a calibration curve and expressed as mg g^{-1} FW^{-1}.

Agronomic parameters

At the maturity stage three plants from each treatment, the combination was collected and agronomic parameters like plant height, siliquae $plant^{-1}$, seeds siliquae^{-1}, 100-seed weight and seed yield $plant^{-1}$ were recorded.

Statistical analysis

The recorded data on various parameters were statistically following MSTAT-C. The treatment means were compared by Least Significant Difference (LSD) test at a 5% level of significance (Gomez and Gomez, 1984).

Result and discussion

Photosynthetic pigment

The accumulation of photosynthetic pigment *viz.* chlorophyll a (Chl a), Chlorophyll b (Chl b) and total chlorophyll (Chl a+b) were significantly influenced due to the imposing of water stress (Fig. 1).

Table 1. Combined effect of irrigation regimes and genotypes on growth, yield and yield attributes of Rapeseed-mustard during *rabi* (winter) season 2017-2018.

Irrigation regimes	Genotypes/varieties	Plant height(cm)	Number of siliquae plant^{-1}	Number of seeds siliqua^{-1}	1000-seed wt (g)	Seed yield plant^{-1} (g)
Control (with Irrigation)	BC-9909	154.3	95	24	3.12	9.32
	BJDH-05	167.2	105	23	3.16	10.34
	Nap-0544	156.8	116	22	3.45	10.22
	BARI Sharisha-11	145.4	86	20	3.10	10.15
	BARI Sharisha-16	139.3	109	19	3.16	9.32
Drought (no irrigation)	BC-9909	110.3	70	15	3.25	6.40
	BJDH-05	124.3	79	18	3.21	8.12
	Nap-0544	115.6	100	20	3.42	8.22
	BARI Sharisha-11	107.7	75	16	3.15	7.68
	BARI Sharisha-16	120.2	89	15	3.12	6.39
CV (%)		5.60	3.53	5.23	0.35	5.63
$LSD_{0.05}$		7.30	8.63	1.23	0.12	2.38

The restricted water supply accumulated (27.5%), (54.5%), 37.5% lowered Chl a, Chl b, total chlorophyll (a+b) respectively over the irrigated condition. However, the negative effect of drought on the accumulation of chlorophyll was more severe for the varieties/genotypes of BC-9909 and BJDH-05. The genotype Nap-0544 and BARI Sarisha-16 accumulated 22.9% and 3.57% lower but BARI

Sarisha-11 accumulated 5.0% higher chlorophyll under drought conditions. Besides, Chl b content decrease over the control was evaluated from 36.0% to 60.0% irrespective of BC-9909, BJDH-05, Nap-0544 genotypes/varieties when subjected to water stress. Among the genotypes, the minimum total chlorophyll Chl (a+b) was reduced in Nap-0544 under drought stress conditions. A decrease of total chlorophyll with drought stress indicates a lowered capacity for light harvesting. Earlier literature like Ommen *et al.* (1999), Upadhyaya *et al.* (2008) and Oneto *et al.* (2016) revealed that drought stress could also reduce the leaf chlorophyll contents, which on the other hand may hamper the photosynthetic efficiency and plant growth.

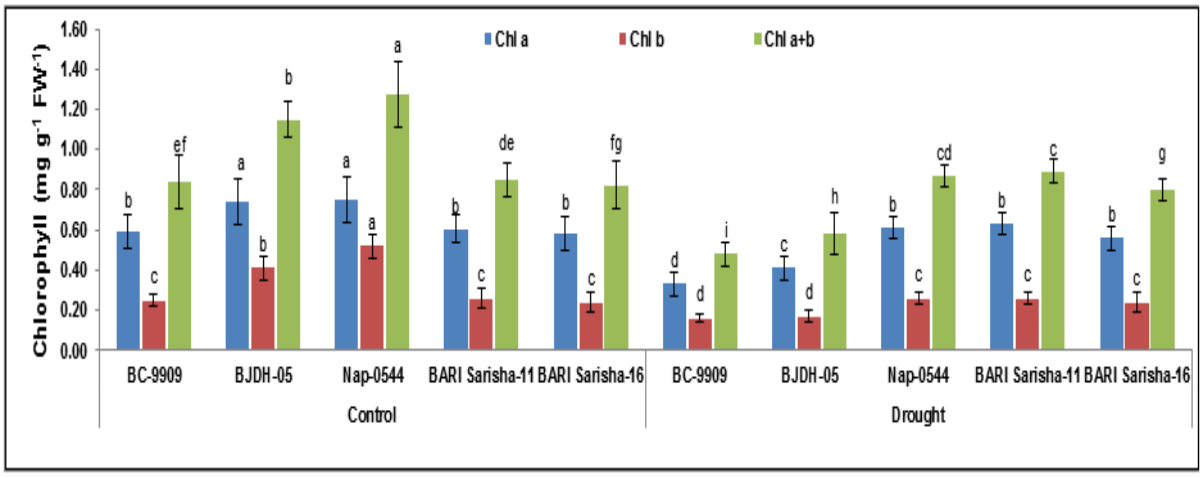

Fig. 1. Accumulation of photosynthetic pigment Chl a, Chl b, Chl a+b of different rapeseed-mustard varieties under drought conditions.

Accumulation of antioxidant enzymes

The antioxidant enzyme activities including CAT, POD and APX were measured to examine whether drought stress inclined the major ROS-scavenging mechanisms in rapeseed-mustard genotypes. Catalase (CAT) detoxifies H_2O_2 formed under extreme water deficit conditions to form water and oxygen (Nahar *et al.* 2018). Generally, an elevated level of CAT activity indicates drought resistance in varieties. Irrespective of genotype, the maximum CAT activity was detected under drought stress conditions. Excepting the genotype BC-9909, rest all others showed the highest CAT activity under drought stress.

The CAT activity was approximately 48.8%, 46.52%, 207.00% higher respectively for genotypes/varieties Nap-0544, BARI Sarisha-11, BARI Sarisha-16 compared with control Fig. 2 (A). Peroxidase (POD) is an enzyme that protects the cells against the destructive influence of H_2O_2 by catalyzing its decomposition through oxidation of phenolic and endiolicco substrates (Lin and Kao, 2002). Under control condition, there were significant differences among POD activities of all the tested genotypes before drought stress with the values of 0.80, 0.54, 0.62, 0.61 and 0.68 mM g^{-1} FW^{-1} protein for genotypes/varieties BC-9909, BJDH-05, Nap-0544, BARI Sarisha-11 and BARI Sarisha-16 respectively.

Results revealed that, the activity of POD decreased in BC-9909 genotype under water stress condition as compared to control while increased in rest all other genotypes. Under drought stress condition, POD activities of increased significantly by 35.2%,163.0%,166.0% and 110.3% in genotypes BJDH-05, Nap-0544, BARI Sarisha-11 and BARI Sarisha-16, respectively Fig. 2 (B). Results of this study indicated that rapid development of higher POD activity under stress should be a trail of tolerant plant species or genotypes, enabling plants to protect themselves against oxidative stress (Zhang *et al.* 2007). Drought tolerance or sensitivity is positively correlated with antioxidant enzyme accumulation in plant genotype.

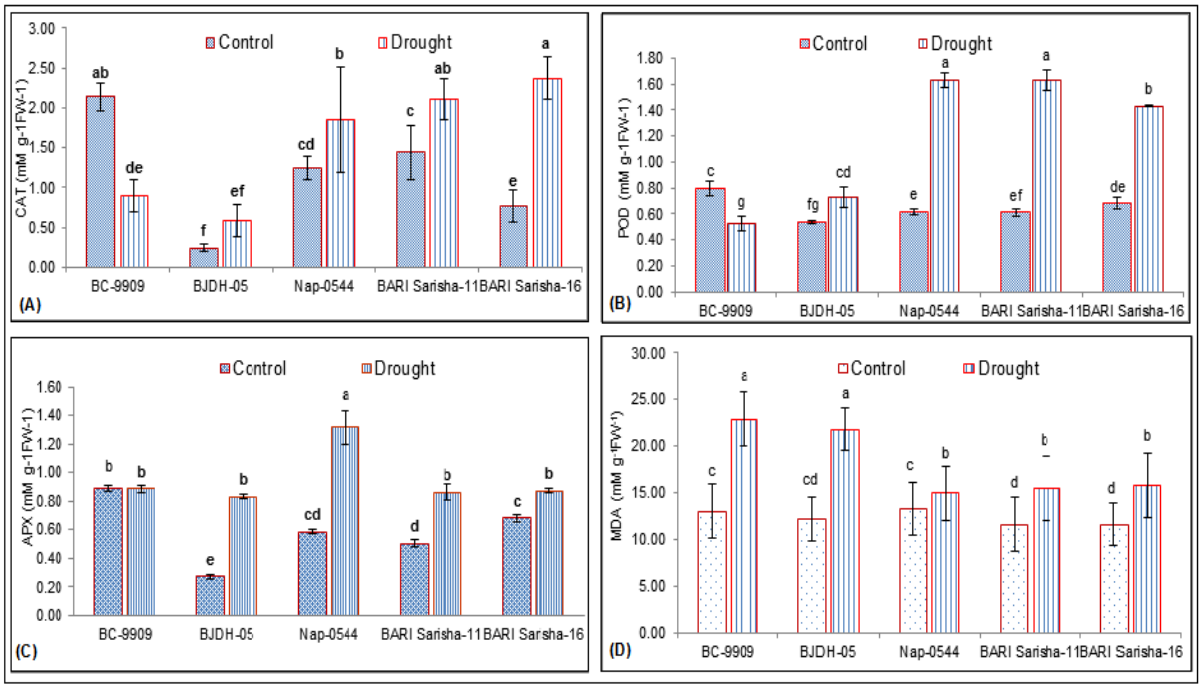

Fig. 2. Effect of drought stress on enzymatic antioxidant (A) Catalase (CAT), (B) Peroxide dismutase (POD), Ascorbate peroxidase (APX), (C) and non-enzymatic antioxidant (D) Malondialdihyde (MDA) activities in Rapeseed-mustard genotypes/varieties comparing with control.

The antioxidant enzyme Ascorbate peroxide (APX) was significantly 63.7% higher under water-stressed conditions (no irrigation) over the control (irrigation) irrespective of genotypes/varieties. Among genotypes/varieties, the highest APX activity was found in genotype Nap-504 followed by the genotype BC-9909. Interactively, the APX activities of genotypes/varieties BC-9909 and BARI Sarisha-16 were comparatively lower in response to drought while an increase of this enzyme activity was seen in BJDH-05 and Nap-0544 Fig. 2 (C). Malondialdehyde (MDA) is regarded as an indicator for evaluation of lipid peroxidation or damage to plasma membranes that increases with the extent of oxidative stress caused by hypoxia or anoxia (Garg and Manchanda, 2009). Hypoxia stress triggers the formation of ROS and induces oxidative stress in plants (Narayanan *et al.* 2005) and by their subsequent reactions, MDA is formed as an oxidation product (Gill and Tuteja, 2010).

In the present study, the content of MDA (lipid peroxidation) strangely increased in all the rapeseed-mustard genotypes under drought conditions compared with respective control Fig. 2 (D). Under drought stress, the MDA content was the highest 76.2% and 79.2% respectively in genotype BC-9909 and BJDH-05. While MDA content was the lowest 12.4%, 33.2% and 35.5% respectively in genotypes Nap-0544, BARI Sarisha-11 and BARI Sarisha-16 Fig. 2 (D).

Proline accumulation

Proline accumulations varied significantly among genotypes, irrigation regimes and the genotype by irrigation regime interactions.

The proline accumulations in the plant tissue increased in all varieties of rapeseed-mustard under drought conditions. Irrespective of genotypes/varieties, the water deficit condition accumulated significantly 91.5% higher proline over the control condition (Fig. 3). Among the genotypes/varieties, Nap-0544 accumulated the highest amount of proline followed by BARI Sarisha-16. Similarly, Qayyum *et al.* (2013) found the genotypic differences in proline accumulation in wheat genotypes exposed to water stress. An elevated level of proline in genotype exposed to stress, implying some levels of osmotic adjustment.

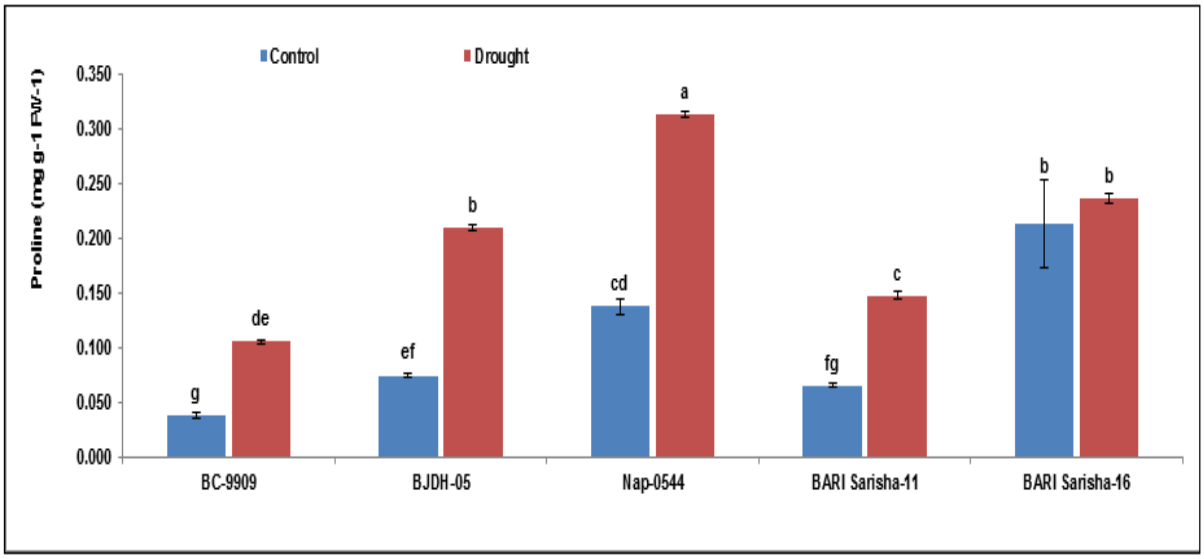

Fig. 3. Proline accumulation of different rapeseed-mustard varieties under drought conditions.

Morphological, Yield and yield contributing parameters

Drought stress conditions hampered the morphological growth like plant height of all genotypes/varieties. Compared with the control plant height was severely (29.0%) reduced in genotype BC-9909 under drought while variety BARI Sarisha-16 was slightly reduced (13.7%) followed by Nap-0544, BJDH-05 and BARI Sarisha-11 (Table 1). The number of siliquae in plant^{-1} reduced with the subjecting of drought stress.

The reduction in siliqua plant^{-1} at drought stress conditions ranged from 12.8% to 26.3%. However, among the genotypes, Nap-0544 produced the highest 86.0% siliqua over control at drought conditions. The production of siliqua plant^{-1} at drought stress reduced to a great extent and ranged from 12.8% in variety BARI Sarisha-16 to 26.3% in genotype BC-9909. Drought also reduced the average number of seeds siliqua^{-1} significantly in all the rapeseed-mustard genotypes studied. The number of seeds siliqua^{-1} was greatly affected by drought in most of the genotypes while genotype Nap-0544 was slightly affected. Under drought stress conditions, the genotype Nap-0544 produced the highest relative seed number siliqua^{-1} which was 91.0% followed by BARI Sarisha-11 (80%) and BARI Sarisha-16 (79.0) and the lowest by genotype BC-9909. For 1000-seed weight (g) the highest was found at Nap-0544 (3.45) while the genotype BARI sharisha-11 gave the lowest (3.10) in control drought. Compared to well-watered in rapeseed-mustard, the grain yield in palnt^{-1} of the water stress rapeseed-mustards decreased by 31.0% in BC-9909 and BARI Sarisha-16 and lowest 19.6% was reduced in genotype Nap-0544.

Conclusion

Present investigation indicated that rapeseed-mustard genotypes were significantly different for various physiochemical traits and yield performance across drought stress. Considering all the physicochemical parameters it might be concluded that genotype Nap-0544 was more drought tolerant than other genotypes. This genotype is proposed for further assessment for varietal endorsement to suggest for general cultivation on farmer fields in drought-affected areas.

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