

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(9): 997-1011
www.biochemjournal.com
Received: 02-07-2024
Accepted: 03-08-2024

Pooja R Kadam
Research Scholar, College of
Agriculture Biotechnology,
Latur, Vasantao Naik
Marathwada Agricultural
University, Parbhani,
Maharashtra, India

Mahendra S Dudhare
Associate Professor, College of
Agriculture Biotechnology,
Latur, Vasantao Naik
Marathwada Agricultural
University, Parbhani,
Maharashtra, India

Uwais S Shaikh
Research Scholar, College of
Agriculture Biotechnology,
Latur, Vasantao Naik
Marathwada Agricultural
University, Parbhani,
Maharashtra, India

Siddhesh S Pawade
Research Scholar, College of
Agriculture Biotechnology,
Latur, Vasantao Naik
Marathwada Agricultural
University, Parbhani,
Maharashtra, India

Corresponding Author:
Pooja R Kadam
Research Scholar, College of
Agriculture Biotechnology,
Latur, Vasantao Naik
Marathwada Agricultural
University, Parbhani,
Maharashtra, India

Studies on morphological and biochemical alterations associated with drought tolerance in linseed

Pooja R Kadam, Mahendra S Dudhare, Uwais S Shaikh and Siddhesh S Pawade

DOI: <https://doi.org/10.33545/26174693.2024.v8.i9m.2318>

Abstract

Water deficit disturbs various physiological and biochemical traits and has adverse bearing on plant growth and productivity. This study aims to explore linseed's response to drought stress and its impact on germination, growth, chlorophyll fluorescence, proline, carbohydrates, and protein profiling. It seeks to enhance understanding of linseed genotype diversity and its correlation with yield for future breeding efforts. In this study, around 32 genotypes were utilized for the evaluation of morphological and biochemical analysis for different traits under two environmental conditions viz., irrigated condition and water-stressed condition. Morphological characterization result indicates that germplasms like RLC-178, RLC-179, RLC-180, RFC-19-1, RLC-177 showed good performance under stressed condition as this germplasms does not show any reduction in all traits. Whereas R-2497, R-2596, R-2598, R-2728, R-3797, R-3986 showed reduction in all morphological trait as indicating susceptibility of germplasms for water-stressed condition. R-2596 (3.89 mg/g) exhibited minimal chlorophyll decreased, suggesting high tolerance, while R-3953 (12.33 mg/g), showed significant decreased, indicating susceptibility to water stress. RLC-177 (27 mg/g), R-2452 (26 mg/g), R-2323 (25 mg/g), R-3751 (25 mg/g), R-3549 (mg/g) showed increased carbohydrate content under water stress, indicating tolerance. RFC-19-2 (25 mg/g), R-2272 (24 mg/g), R-2281 (12 mg/g), R-3785 (12 mg/g), and (R-3977 (11 mg/g) show decreased carbohydrate content under water stress, indicating susceptibility to water stress condition. R-2323 (11.89 μ mole/gm) shows a high increased in proline content under stress condition. Protein profiling using SDS-PAGE gel electrophoresis showed total eight scorable bands were recorded under the irrigated condition and six scorable bands were obtained under stressed conditions. Overall, the research indicates low level genetic variation in the total seed protein profiles analyzed within linseed germplasms.

Keywords: Chlorophyll, proline, SDS-PAGE gel electrophoresis

Introduction

Linseed also referred to as flax (*Linum usitatissimum* L.), is a self-pollinated crop widely adapted to temperate climates of the world. It is an annual plant belonging to the genus *Linum* and the family Linaceae having 14 genera and over 200 species [4]. The linseed's genome measures between ~368-373 Mb [29]. The name *Linum* is derived from the Celtic word *lin*, which means "thread", and the name *usitatissimum* is Latin for "most useful". The terms flax and linseed have particular meanings, depending on the region. There are two morpho types of *L. usitatissimum*, "flax type" and "linseed type" [9, 17]. In Europe, flax refers to the seed grown for fibre (linen) production, while linseed refers to oilseed flax grown for industrial and nutritional uses [4]. The crop is predominantly self-pollinated, but out-crossing (less than 2%) occasionally results from insect activity [36]. Based on the diversity of plant types, linseed has two centers of origin i.e., South West Asia, particularly in India [38, 30] and the Mediterranean region of Europe [6].

Linseed is rich in fat, protein, and dietary fibre. It contains 40% oil, 30% fibre, 20% protein, 6% moisture, and 4% ash [41, 9]. Flax seeds are rich source of omega fatty acids and in addition provide essential nutrients such as vitamin B6, magnesium, and foliate. Linseed oil is rich in alpha-linolenic acid (ALA), an essential fatty acid beneficial for treating several illnesses, including cancer, rheumatoid arthritis, depression, coronary heart disease, and Crohn's disease [33].

Linseed's high linolenic acid offers anti-ulcer, antioxidant, anti-carcinogenic, anti-inflammatory properties, commonly used in industrial processes like paint production [39]. Like many other field crops, flax also faces abiotic stresses during its growth period. Among various abiotic stresses, hydropenia i.e., drought stress is the most detrimental stress inhibiting the growth and development of flax at vegetative as well as reproductive stage [24]. Drought stress also diminishes leaf expansion and production and promotes senescence and abscission [15]. Plants can adjust to water stress by changing the metabolism of their cells, thereby triggering different defence systems [10]. It involves the synthesis and accumulation of small compatible solutes (Osmolytes), such as proline, glycine betaine, sugars, and specific inorganic ions [5]. Proline accumulation is a notable metabolic response of plants to drought stress conditions [21]. Linseed is an important cultivated crop; it is very much required to conserve the genetic material of linseed from as wide resources as possible for future breeding efforts. To maintain and exploit the genetic resources, it is required to understand the amount and distribution of genetic variation within and among accessions. In this study, we aim to investigate the response of linseed to drought stress and the effects of water stress on the germination and growth of linseed seedlings, including effects on chlorophyll fluorescence and on accumulations of proline, carbohydrates, and analysis of protein profiling.

Materials and Methods

Material (Germplasm)

The linseed genotypes for the present study were obtained from the germplasm maintained at Indira Gandhi Agricultural University, Raipur (C. G.) through Material Transfer Agreement (MTA) and screened for *in-vitro* drought tolerance in the year 2020-2021. Around 32 genotypes were utilized for the evaluation of morphological and biochemical analysis for different traits during the present investigation. These are: RLC-176, RLC-177, RLC-178, RLC-179, RLC-180, RFC-19-1, RFC-19-2, R-2272, R-2281, R-2323, R-2452, R-2497, R-2596, R-2598, R-2728, R-3368, R-3396, R-3545, R-3549, R-3751, R-3768, R-3785, R-3793, R-3797, R-3838, R-3944, R-3953, R-3977, R-3986, R-4028, RLC-173, RLC-175.

Conduct of field experiment

The experiment was laid in randomized block design (RBD) at the Oilseed Research Station, Latur (M.S.) India, during the Rabi season of 2022. Selected germplasm lines grown at two different plots viz., Normal condition and water stressed condition with two replications of each plot. In each replication, the genotypes were grown in 2 m long rows with spacing of 30 cm between rows and 10 cm between the plants. Within a row, seeds were hand-dibbled 10 cm apart. A fertilizer dose of 40 Kg N:20 Kg P:5 Kg K per hectare was applied at the time of sowing. A standard package of practices was followed to raise the good crop.

Drought imposition

As linseed is rabbi crop, I irrigation was given at the time of sowing and II irrigation was given before the flowering stage for both water stressed and normal condition plot. After flowering no irrigation was given to the stressed condition plot while one extra irrigation was given to the

normal condition plot. Stressed condition imposed after 50% flowering.



Fig 1: General view of experimental plot under normal condition

Methodology

Morphological observations were recorded on five randomly selected plants in each entry from each replication in normal and water stressed conditions for different agronomical traits. Following morphological characters were recorded: Plant height (cm), Number of capsules per plant, Number of seed bearing capsules per plant, Capsule length (cm), Capsule fertility (%), Seed yield per plant (g), 1000 Seed weight (g), Seed length (mm), Seed width (mm), Seed length width ratio, Total root length (cm). Leaf rolling score was recorded for three days continuously under water stress imposed after 50% flowering. The leaf rolling score was calculated using the evaluation system given by (IRRI, 1980). Leaf relative water content was determined by using the procedure given by Merah, 2001 [22].

Biochemical Characterization

Estimation of important biochemical components viz, chlorophyll, carbohydrate, proline, total protein and protein extraction quantification, and SDS electrophoresis was carried out using various specified methods.

Chlorophyll estimation

The standard method for determination of chlorophyll in the laboratory was utilized and chlorophyll-a, chlorophyll-b, and total chlorophyll were determined. Leaves from irrigated and water stress samples were washed with distilled water, dried with filter paper, and pulverized. The resulting leaf material was homogenized with 80% acetone, and the solutions were transferred to test tubes, adjusting the volume to 10.0 ml with 80% acetone. After centrifugation at 5000 rpm for 10.0 min, the supernatant was collected in separate test tubes, repeating the process until the pellet became clear. Absorbance at 663 and 645 nm was recorded using a UV spectrophotometer for further analysis.

Chlorophyll a, b, and total chlorophyll were calculated by using the following formula:

$$\text{Chlorophyll a (mg gm}^{-1}\text{)} = [12.7 (\text{OD663}) - 2.69 (\text{OD645})] \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b (mg gm}^{-1}\text{)} = [22.9 (\text{OD645}) - 4.68 (\text{OD663})] \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll (mg gm}^{-1}\text{)} = [20.2 (\text{OD645}) + 8.02 (\text{OD663})] \times \frac{V}{1000 \times W}$$

Carbohydrate estimation

The total carbohydrate content of plant tissue was estimated by the Anthrone method suggested by Jayaraman, 1981 [14]. The Anthrone reagent was prepared as per the procedure given by Trevelyan and Harrison, 1952 [37]. Dry and finely powdered 100 mg seed samples were hydrolyzed for 3 hours at 80-85 °C with 5.0 ml of 2.5N HCl. The neutralized supernatant, adjusted to 100 ml with distilled water, was centrifuged at 8,000rpm for 5.0 minutes at 4 °C. A 0.5 ml aliquot was then transferred to a fresh test tube. Additionally, various aliquots from a glucose standard solution, along with sample tubes, were adjusted to a final volume of 1.0ml with double-distilled water, heated, cooled, and assessed for color development at 620nm using a spectrophotometer, with quantification based on a calibration curve. The carbohydrate content in the sample was calculated using the provided formula.

$$\text{Carbohydrate in sample (\%)} = \frac{\text{Sugar value from graph (mg)}}{\text{Aliquot sample used (1.0 ml)}} \times \frac{\text{Total value of extract (ml)}}{\text{Wt. of sample (mg)}} \times 100$$

Proline estimation

The protein content was estimated using the procedure given by Bates *et al.* 1973 [3] using the Acid Ninhydrin method. Fresh leaf samples (0.5 g) from water-stressed and irrigated conditions were crushed with 10 ml of 3% aqueous sulfosalicylic acid, centrifuged at 5000 rpm for 10min, and 2 ml of supernatant treated with glacial acetic acid and acid-ninhydrin reagent. After a one-hour water bath (100 °C) and cooling in an ice bath, agitation with 4 ml of toluene yielded an upper chromophore layer, exhibiting a pinkish-red hue, isolated for absorbance measurement at 520nm, using toluene as a blank

Statistical Analysis: The proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows:

$$\mu\text{moles of proline per gram tissue} = \frac{(\mu\text{g Proline per ml}) \times (\text{ml toluene})}{115.5} \times \frac{5}{\text{gram of sample}}$$

The analysis of variance for individual characters was carried out using the mean values of each plot following the method given by Panse & Sukhamte, 1954 [27]. The data recorded was analyzed statistically using OPSTAT software. The standard error of difference was calculated at 5% probability level to compare the mean differences among the treatments. The differences between the means were compared using Duncan's Multiple Range Test (DMRT $p < 0.05$).

Total seed protein extraction and profiling using SDS-PAGE gel electrophoresis

Protein profiling of linseed samples were analysed through SDS-PAGE electrophoresis as per the protocol described by

Laemmli, 1970 [20]. Protein extraction and purification was done as per the methods given by Schwenk, 1980; Scheller, 2001 [35, 34]. Mature seeds (100 mg) from irrigated and water-stressed conditions were finely powdered and homogenized in 1x phosphate buffer. The homogenized samples were incubated at -20 °C for 1-2 hours, followed by room temperature stabilization for 10 minutes and centrifugation at 10,000 rpm for 10 minutes at 4 °C. The resulting supernatant, collected in fresh tubes, was utilized for subsequent protein profiling through SDS-PAGE Electrophoresis. After that 80µl extracted protein samples were mixed with 20µl of loading buffer in a micro centrifuge tube. The resulting mixture was denatured by boiling at 95-99 °C for 5 minutes, ensuring thorough protein-SDS interaction. Assembling of the SDS-PAGE gel casting apparatus was done as per the procedure given by Simpson, 2006 [33]. Protein profiling was done by using stacking gel (5%) and separating gel (15%). Protein samples, including a marker, were loaded, and the gel was subjected to electrophoresis unit at 30mA for 10 minutes, followed by a reduction to 20 mA for protein separation. The gel was then stained overnight, destained, and banding patterns were analysed using a gel documentation system and a pre-stained protein marker.

Result and Discussion

Analysis of Variance

Analysis of variance showed highly significant differences among the genotypes for all the morphological characters. The differences were observed between irrigated and water-stressed conditions. (*) represents the highly significance value among irrigated condition. Whereas (**) represents the highly significance value among water-stressed condition.

Morphological characterization

The mean performance of 32 linseed germplasm for 13 morphological characters was recorded. The tallest plant height of 75 cm (R-2596) and smallest plant height 36 cm (RLC-176) with mean of 57.86 cm was observed under irrigated condition. The tallest plant height of 66 cm (R-2596) and smallest plant height of 30cm (R-3797) with mean of 46.59cm was observed under stressed condition. R-3751 showed low difference in plant height (0.7cm) between irrigated and water stressed condition, indicating high tolerance; R-3953 exhibited high susceptibility to stressed condition by showing high difference for plant height (20 cm) (Fig. 3). The results of the current findings were similar to the study conducted by Kariuki *et al.*, 2016 [16]. The result of the experiment revealed that water stress led to a significant decrease in the height of the different linseed varieties. Several prior investigations focusing on different crops such as rice [7], maize [28, 13], and safflower [23] have reported a decrease in plant height as an effect of water stress. In irrigated conditions, R-3986 had the highest seed yield per plant (14.54 gm) and R-3396 had the lowest (1.61 gm). Under stress, R-3986 still had the highest yield per plant (7.72 gm) while R-2598 had the lowest (0.94 gm). RLC-177 showed minimal difference (0.16 gm) between stressed and normal conditions. In contrast, R-3986 exhibited a substantial difference (6.82 gm) between stressed and unstressed conditions (Fig 4). Similar results were obtained by Davatgar *et al.* 2009 [7] reductions in rice yield under water stress. In irrigated conditions, R-176 had

the highest 1000 seed weight (9.52 gm) and R-2323 had the lowest (5.20 gm). Under stress, R-3977 had the highest seed weight (9.13 gm) and R-2497 had the lowest (4.25 gm). R-3549 showed minimal reduction in seed weight under stress (Fig 5). Pande *et al.*, 1970^[26] and Kiani *et al.*, 2020^[18] also found a decrease in the weight of 1000 seeds as water availability decreased in different linseed accessions. Germplasms RLC-176, RLC-179, R-3768, RLC-180, RFC-19-1, R-3368, and R-3549 shows no any reduction in seed length under stressed condition (Fig 6). Similar results were found by Kiani *et al.*, 2020^[18] that no significant alterations observed in the length of both seeds and leaves under stressed condition. R-3549 and R-2452 shows high reduction in seed width where some germplasm shows no any reduction in seed width under stressed condition (Fig 7). Under normal condition seed length width ratio varies from 2.3 to 1.6 with a mean of 1.9. The maximum ratio observed in RLC-177, RLC-178, RLC-175. Under stressed condition range of seed length weight ratio varies from 2.5 to 1.7 with average of 2.04. The maximum ratio was observed in R-3838 (2.5), R-2598, R-177 (Fig. 8). Under normal condition the maximum and minimum number of capsules per plant was observed in R-3986 (170) and R-3396 (22) respectively. Under stressed condition the maximum and minimum number of capsules per plant was observed in R-3986 (114) and R-3396 (19) respectively. The maximum reduction of 70 capsules was observed in R-2596 showing susceptibility of germplasm to stressed condition (Fig 9). Similar results were obtained by Davatgar *et al.* 2009^[7] in rice (*Oryza sativa* L.) to different levels of water stress. Under irrigated condition the maximum and minimum number of seed bearing capsules per plant was observed in R-3986 (166) and R-3396 (22) respectively. Under stressed conditions the maximum and minimum number of seed bearing capsules per plant was observed in R-3986 (108) and R-3396 (17) respectively (Fig 10). Under irrigated condition, 100% capsule fertility was observed in RFC-19-1, R-3396, R-3838, R-3944 and 94.6% capsule fertility observed in RLC-178. Under stressed condition, the maximum and minimum capsule fertility was observed in R-3944 (98.6%) and R-2728 (85%) respectively (Fig 11). Under normal conditions, maximum and minimum root length was observed in R-3785 (9.1 cm) and R-2596 (2.9 cm). Under stressed conditions, maximum and minimum root length was observed in R-2272 (9.4 cm) and R-2596 (3.2 cm) respectively (Fig. 2 and Fig. 12). Similar results were obtained in various crops, including chickpea^[1], pea^[11], black gram^[2], rice^[19], and maize^[25]. Under normal conditions, the maximum and minimum capsule length was observed in R-2272 (9.5 mm) and R-3838 (6 mm) respectively. Under stressed conditions, the maximum and minimum capsule length was observed in R-3396 (9 mm) and R-2281 (5 mm) respectively (Fig 13). Under normal conditions, R-2497, R-4028, and R-3785 showed slight leaf tip drying on Day 3, scored 1. Under stress conditions, R-3785 exhibited 50% full dried leaves on Day 3, scored 5, while R-2497, R-2598, and R-2728 had tip drying extending to ¼ length in 50% leaves with 25% full leaf drying, scored 4. Similar results were obtained by Datta *et al.*, 1988^[8] in rice. Under irrigated conditions, the maximum and minimum leaf relative water content was observed in R-2596 (76.69%) and RLC-176 (56.1%) respectively. Under stressed conditions, the maximum and minimum leaf

relative water content was observed in R-3549 (65.55%) and R-3768 (42.30%) respectively (Fig 14).

Biochemical characterization

Chlorophyll estimation

Under normal conditions, the highest and lowest chlorophyll content was observed in RLC-177 (14.19 mg/g) and R-2596 (8.51 mg/g) respectively. Under stressed conditions, the highest and lowest chlorophyll content was observed in R-3751 (10.10 mg/g) and R-2598 (0.74 mg/g) respectively. R-2596 (3.89 mg/g) shows a very low amount of decrease in chlorophyll content over normal conditions indicating high tolerance. Whereas high level of decrease in chlorophyll content was observed in R-3953 (12.33 mg/g) showed susceptibility of variety for water stress (Fig. 15).

Carbohydrate Estimation

Under normal conditions, the highest and lowest carbohydrate content was observed in RLC-173 (98 mg/g) and RLC-179 (31 mg/g) respectively. Under stressed conditions, the highest and lowest carbohydrate content was observed in RLC-173 (101 mg/g) and RFC-19-2 (22 mg/g) respectively. From these findings, it was concluded that carbohydrate content in RLC-177 (27 mg/g), R-2452 (26 mg/g), R-2323 (25 mg/g), R-3751 (25 mg/g), R-3549 (mg/g) increase over the normal condition. While carbohydrate content in RFC-19-2 (25 mg/g), R-2272 (24 mg/g), R-2281 (12 mg/g), R-3785 (12 mg/g), and (R-3977 (11 mg/g) decrease over normal conditions (Fig 16).

Proline Estimation

Under normal condition, the maximum and minimum proline content was observed in R-3793 (9.56 µmole/gm) and RLC-176 (5.12 µmole/gm) respectively. Under stressed condition, the maximum and minimum proline content was observed in RLC-180 (19.52 µmole/gm) and R-3768 (11.25 µmole/gm) respectively. R-2323 (11.89 µmole/gm) shows a high increase in proline content over normal conditions. Conversely, the germplasm R-2598 showed very little increase in proline content (2.04 µmole/gm) over the normal conditions. Guo *et al.*, 2012^[12] found that as PEG concentration increased, chlorophyll content decreased. They also noted that water stress induced by PEG led to increased concentrations of carbohydrates and proline in shoots and roots of linseed seedlings (Fig. 17).

Protein profiling using SDS-PAGE Gel Electrophoresis

The total protein present in linseed was determined by using the protocol described by Laemmli 1970^[20]. On the basis of SDS-PAGE gel electrophoresis, bands were scored for presence (1) or absence (0). Clustering and similarity matrix were carried out by using UPGMA using NTSYSpc-2.02i software. A total eight scorable bands were recorded under the irrigated condition (Fig No. 18) and six scorable bands were obtained under stressed conditions (Fig No. 19). The size (molecular weight) of polypeptide bands produced by SDS-PAGE identified by protein molecular weight marker ranges from 20 to 118 kDa. The size of bands ranges between 1.5 kDa to 118 kDa. Out of a total 8 bands under irrigated conditions, 7 bands were polymorphic and 1 band was monomorphic. Under normal conditions, the highest band size of ~60 kDa was present in R-3549 and R-3751. Lowest band size of 1.5 kDa was observed. The size of bands were different viz. band 3 (~35 kDa), band 4

(26~kDa), band 5 (~24 kDa), band 6 (20 kDa), band 7 (~10 kDa), and band 8 (~1.5 kDa). The band 6 (20 kDa) was common in all genotypes. Whereas band 1 (~60 kDa) and 2 (~40 kDa) was absent in all genotypes, except R-3549 and R-3751. Out of a total 6 bands under stressed conditions, 5 bands were polymorphic and 1 band was monomorphic. Under stressed conditions, the highest band size of ~35 kDa was present in R-3549 and R-3751. Lowest band size of 1.5 kDa was observed. The results of the study are similar with the findings of Sardar *et al.*, 2012 ^[32]. They analyzed total seed protein pattern of rice. Out of 16 observed protein bands, 81% were found to be polymorphic, while 19% were monomorphic, exhibiting molecular weights between 100 and 120 kDa. Two universal bands (5 and 6) were identified across all genotypes. The similarity coefficients, ranging

from 0.67 to 1.00, indicated varying degrees of similarity among genotypes. Cluster analysis via UPGMA procedure under normal condition exposed two major clusters (Cluster A and Cluster B) at 60% similarity (Fig No. 20). While under stressed condition exposed two major clusters (Cluster A and Cluster B) at 73% similarity (Fig No.21). Overall, the study suggests a low level of genetic diversity in the total seed protein profiles of the examined linseed samples. Similar results were obtained by Sammour *et al.*, 1999 ^[31] in linseed. They revealed that the protein electrophoretic profiles, obtained by extracting proteins using water, buffer, urea, and SDS and subjecting them to SDS-PAGE and Poro-SDS-PAGE without reducing agents, revealed six predominant bands with molecular weights of 55kDa, 50kDa, 47kDa, 45kDa, 43kDa, and 41kDa.

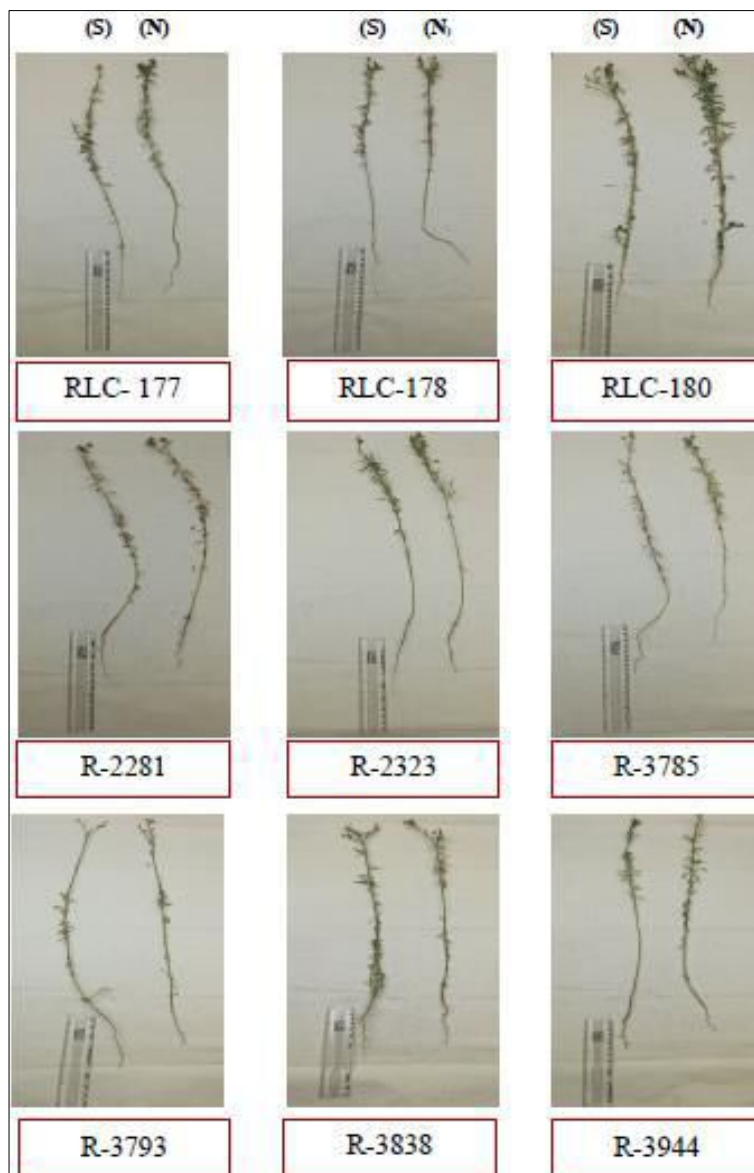


Fig 2: Variation in total root length between normal and stressed condition germplasms (N)- Normal condition, (S)- Stressed condition

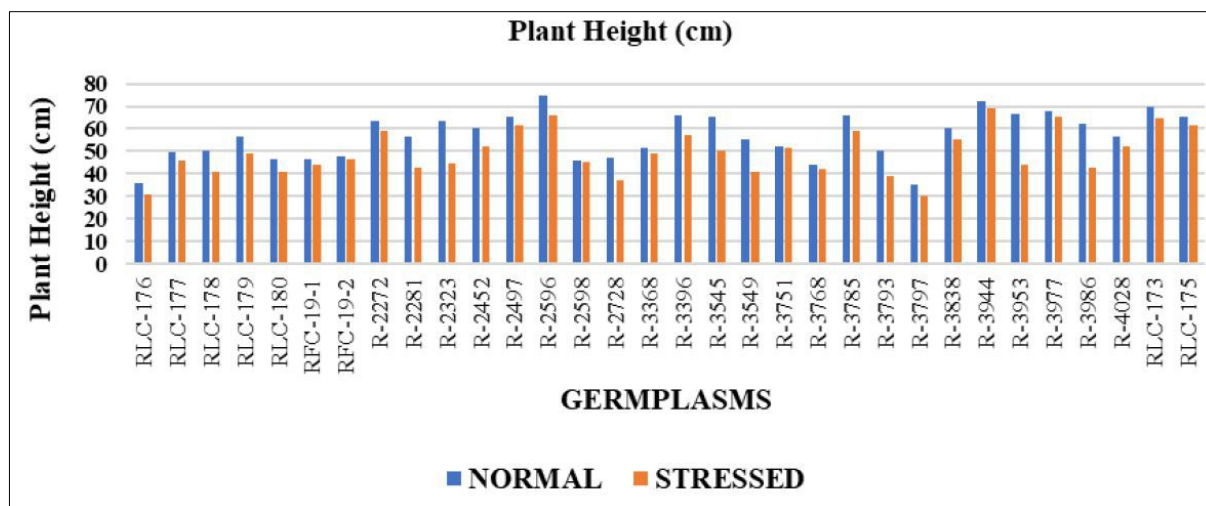


Fig 3: Plant height of 32 linseed genotypes under normal and stressed conditions

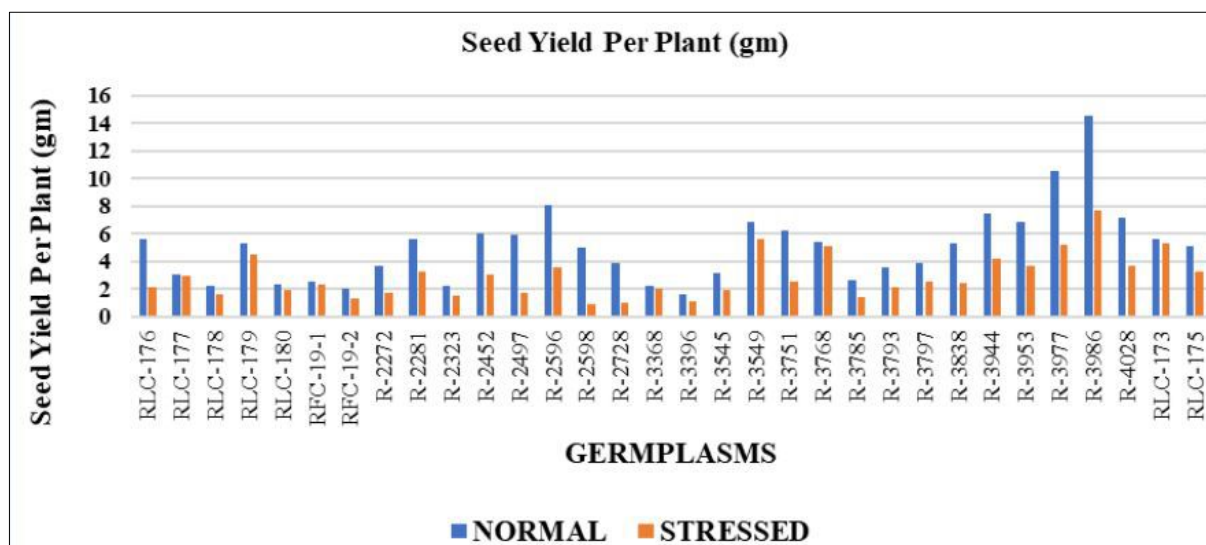


Fig 4: Seed yield per plant of 32 linseed genotypes under normal and stressed conditions

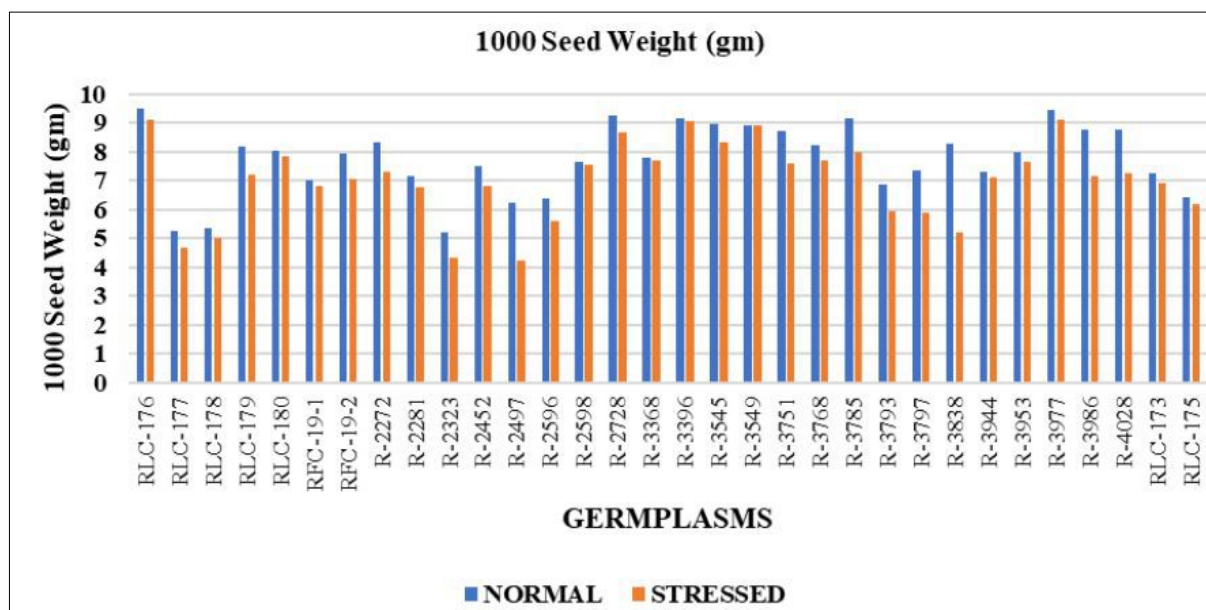


Fig 5: 1000 seed weight of 32 linseed genotypes under normal and stressed conditions

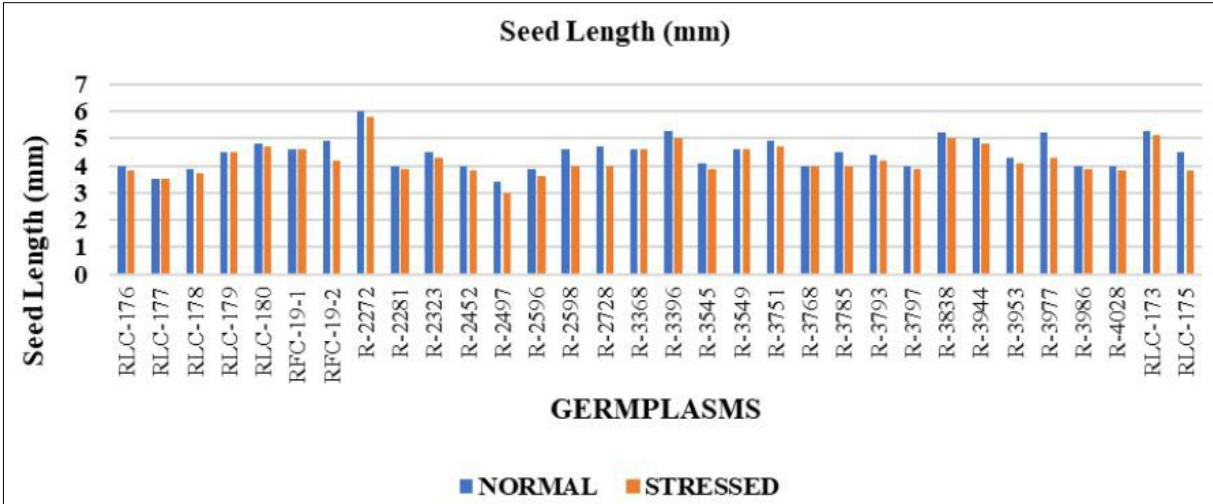


Fig 6: Seed length of 32 linseed genotypes under normal and stressed conditions

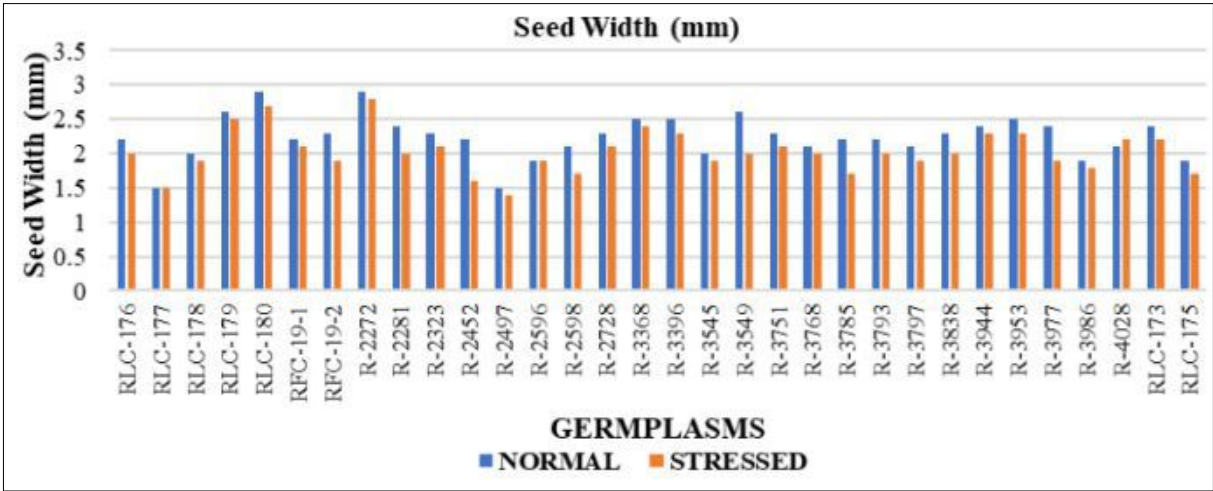


Fig 7: Seed width of 32 linseed genotypes under normal and stressed conditions

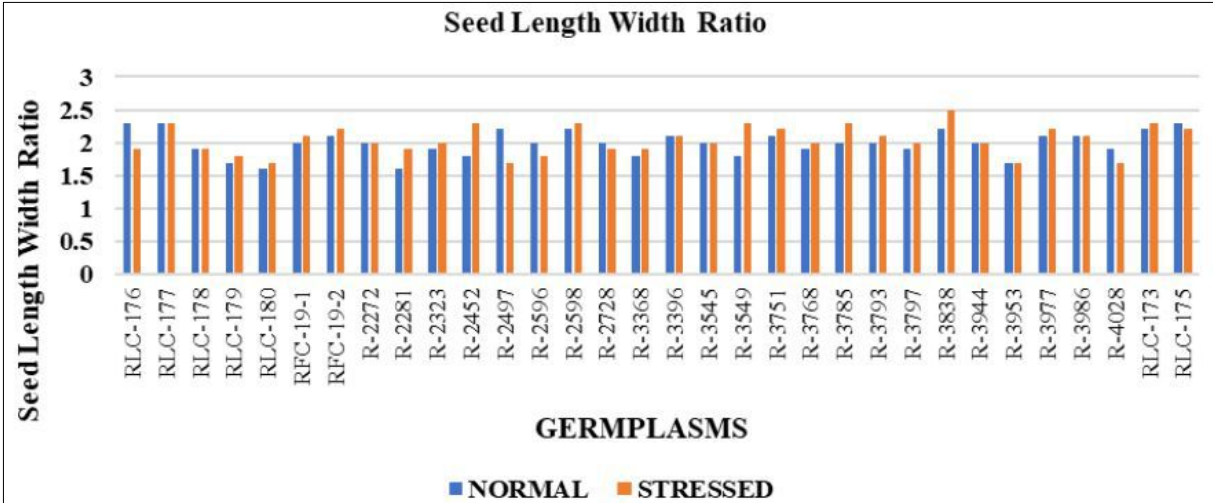


Fig 8: Seed length width ratio of 32 linseed genotypes under normal and stressed conditions

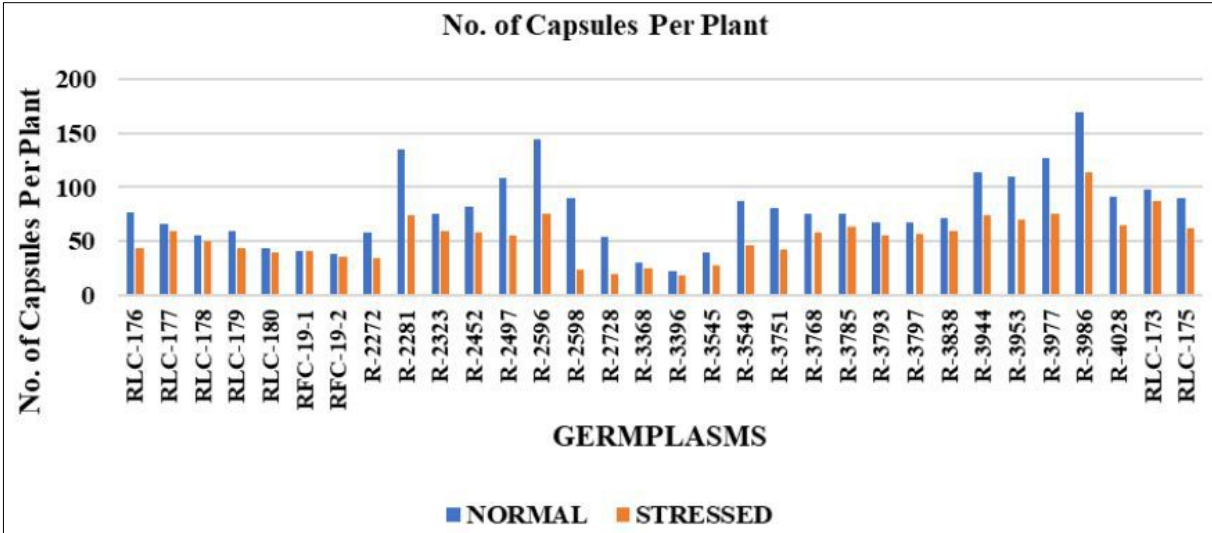


Fig 9: Number of capsules per plant of 32 linseed genotypes under normal and stressed conditions

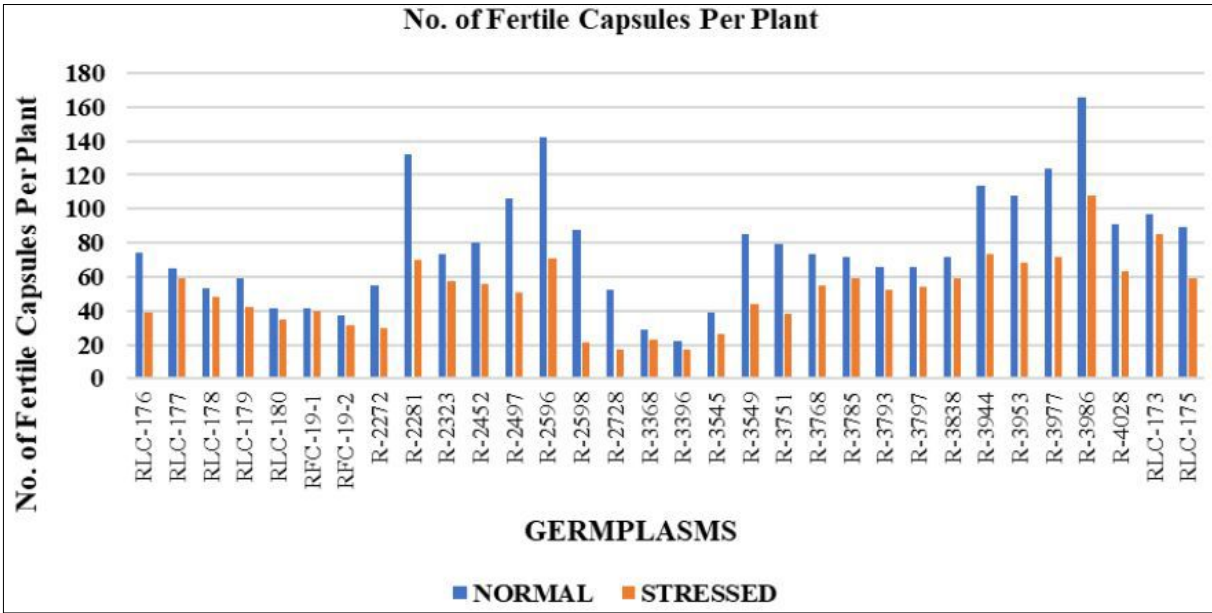


Fig 10: Number of seed-bearing capsules per plant of 32 linseed genotypes under normal and stressed conditions

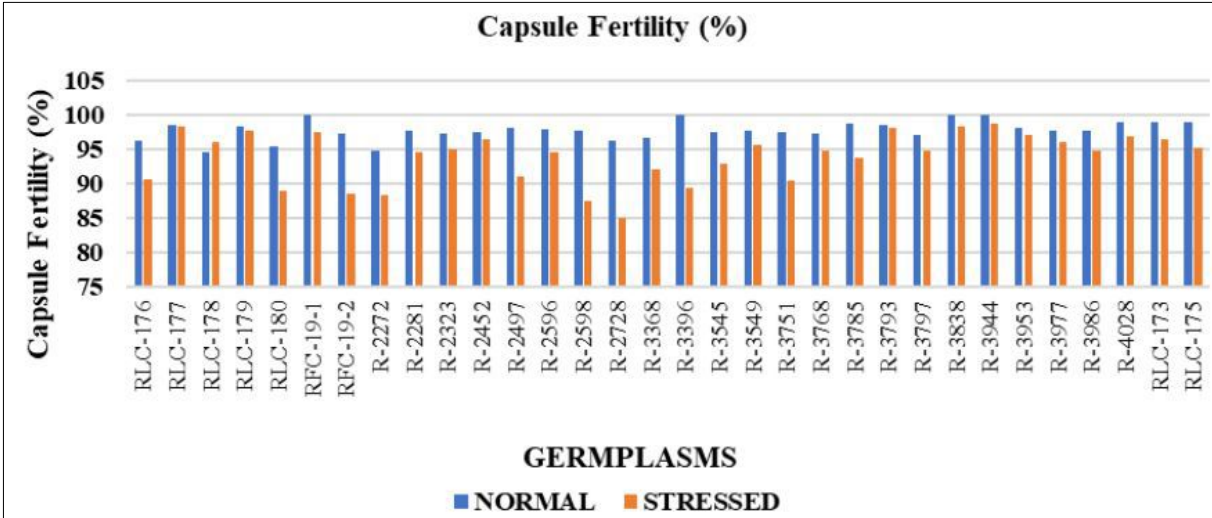


Fig 11: Capsule fertility of 32 linseed genotypes under normal and stressed conditions

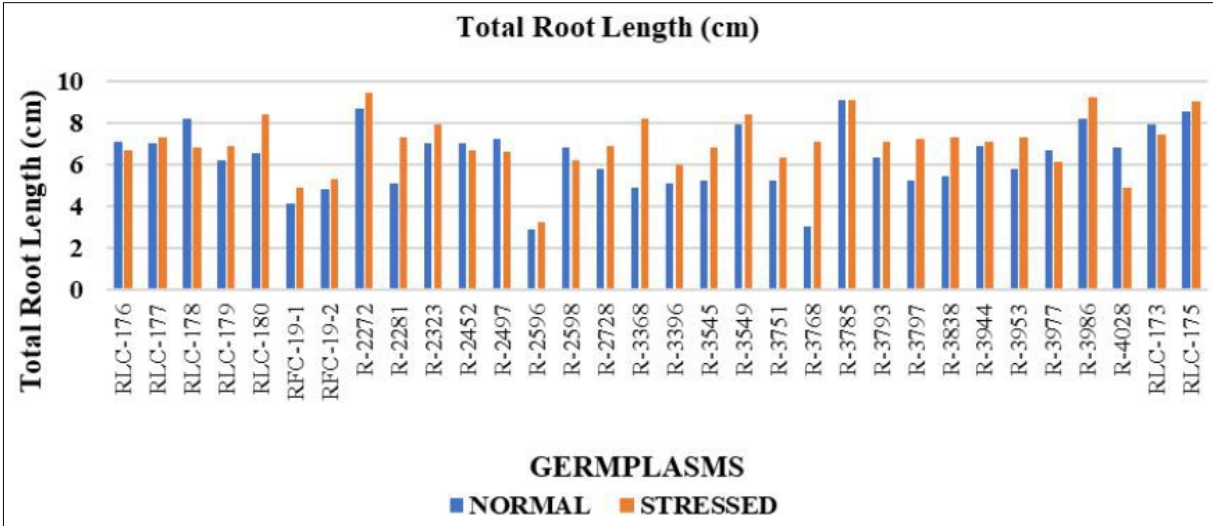


Fig 12: Total root length of 32 linseed genotypes under normal and stressed conditions

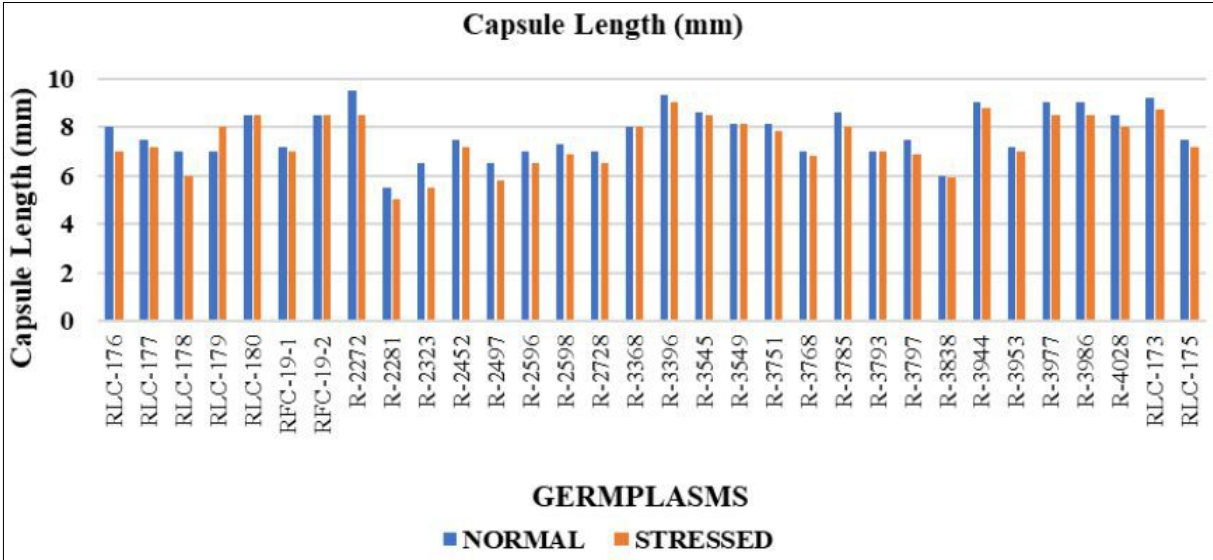


Fig 13: Capsule length of 32 linseed genotypes under normal and stressed conditions

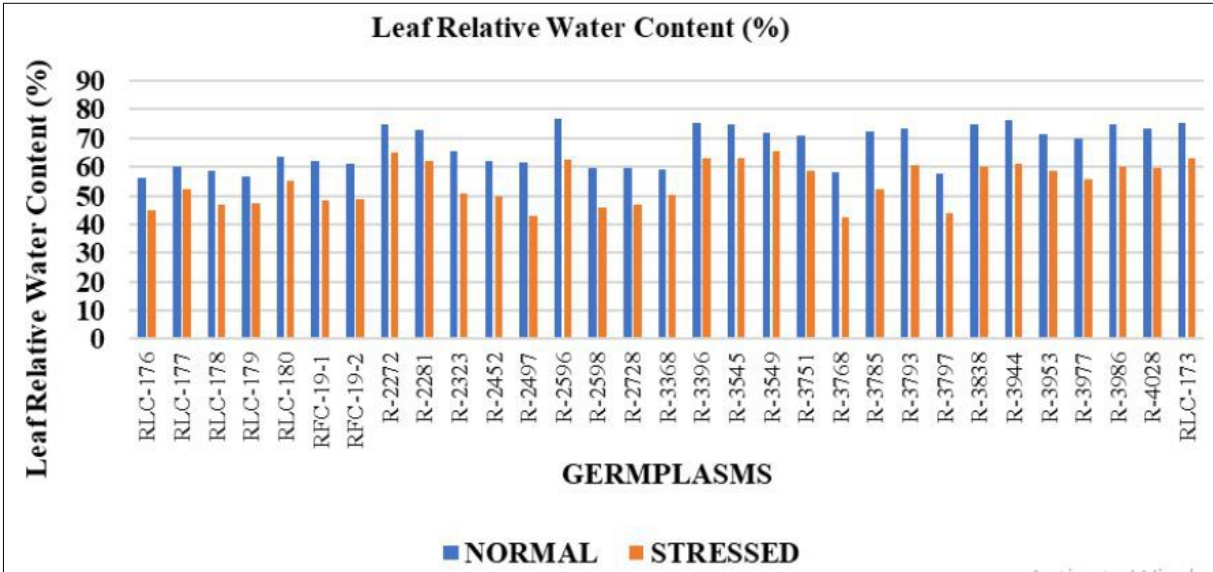


Fig 14: Leaf relative water content of 32 linseed genotypes under normal and stressed conditions

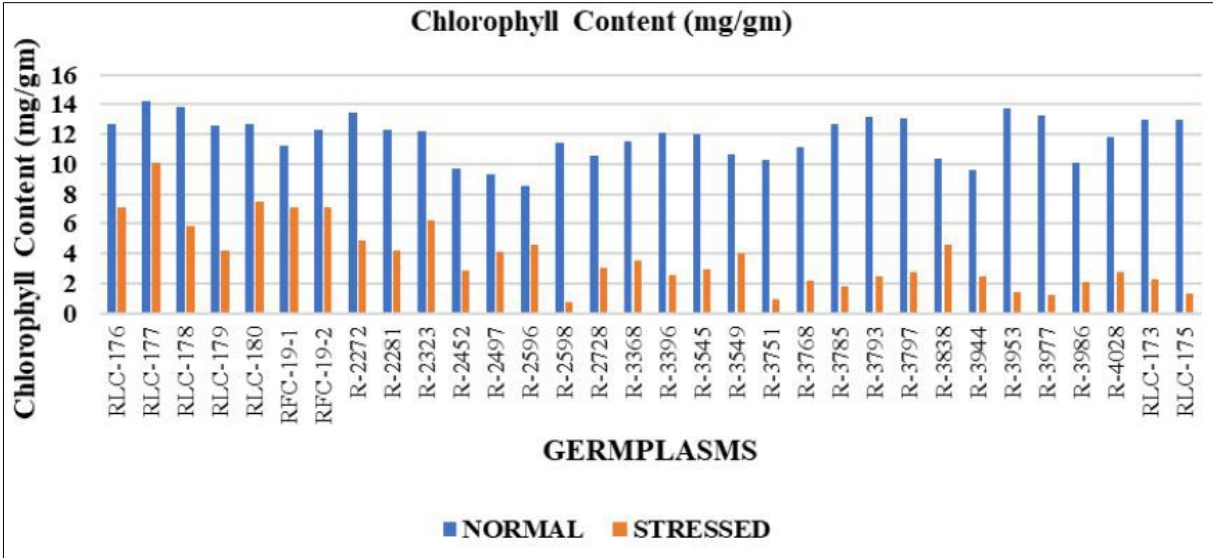


Fig 15: Chlorophyll content of 32 linseed genotypes under normal and stressed conditions

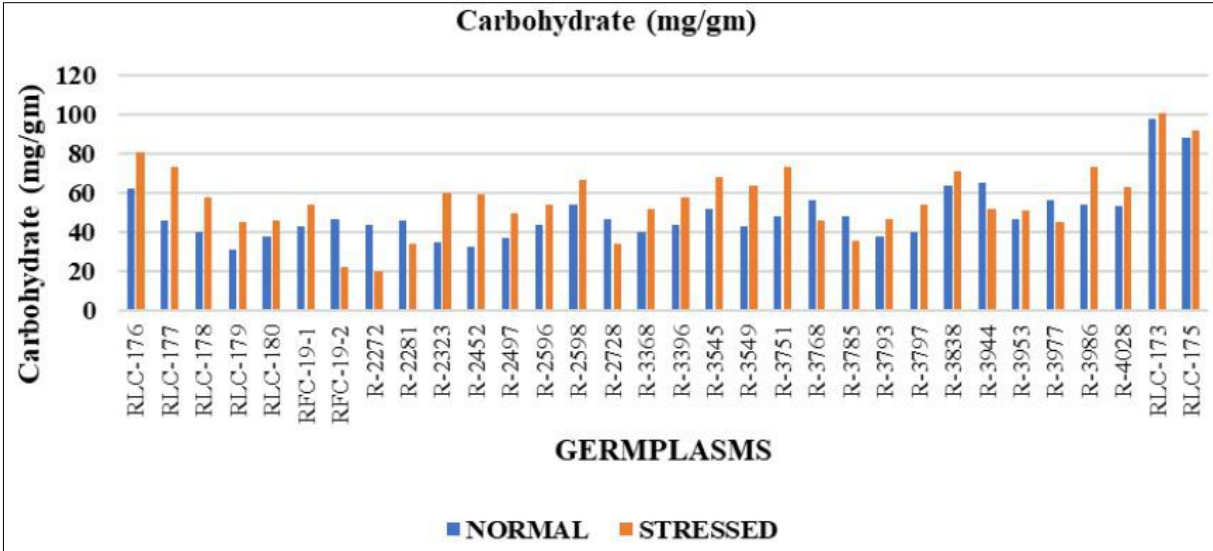


Fig 16: Carbohydrate content of 32 linseed genotypes under normal and stressed conditions

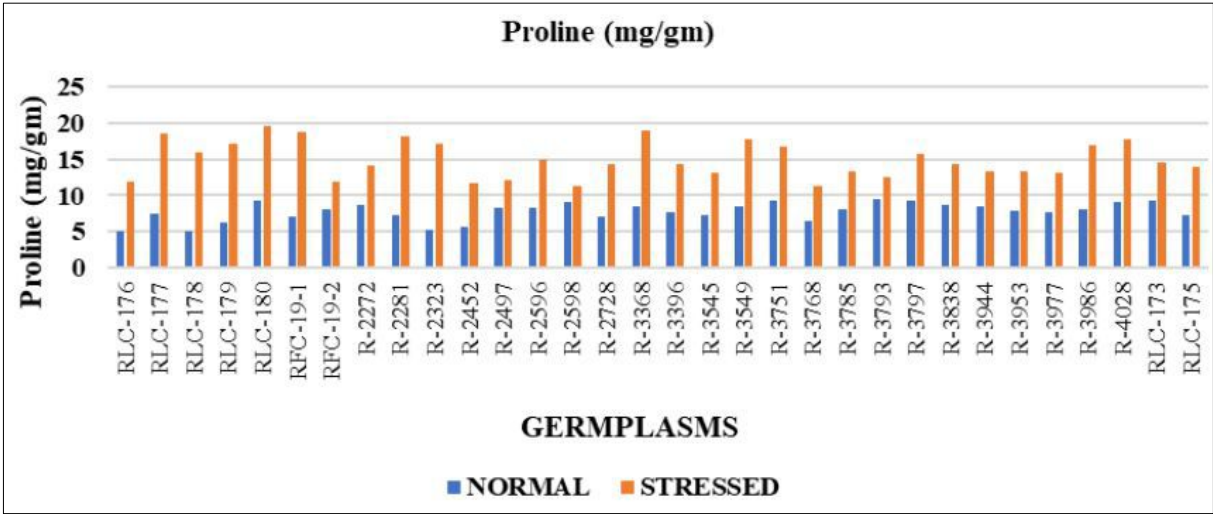


Fig 17: Proline content of 32 linseed genotypes under normal and stressed conditions

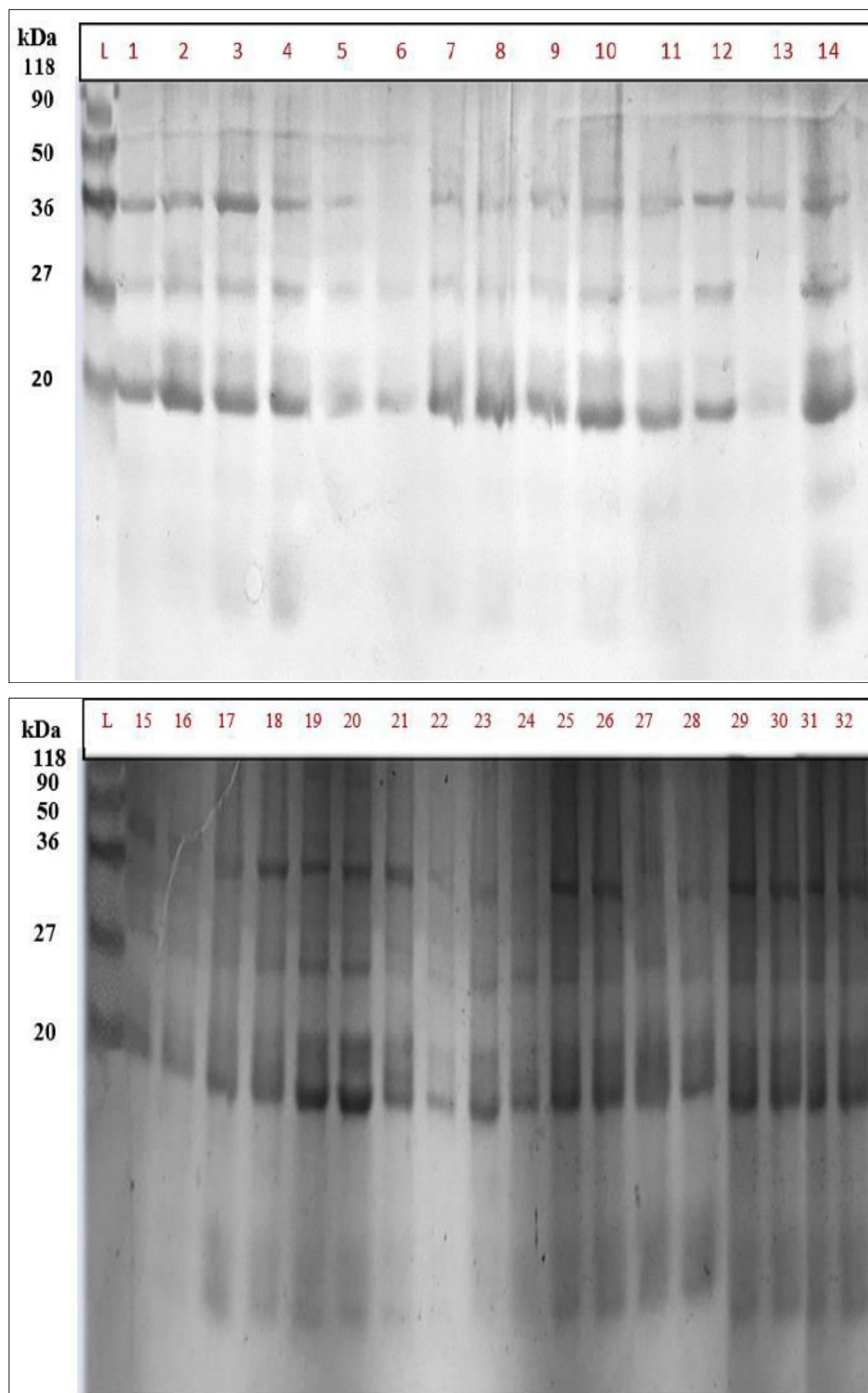


Fig 18: Protein profiling of 32 linseed genotypes under normal condition

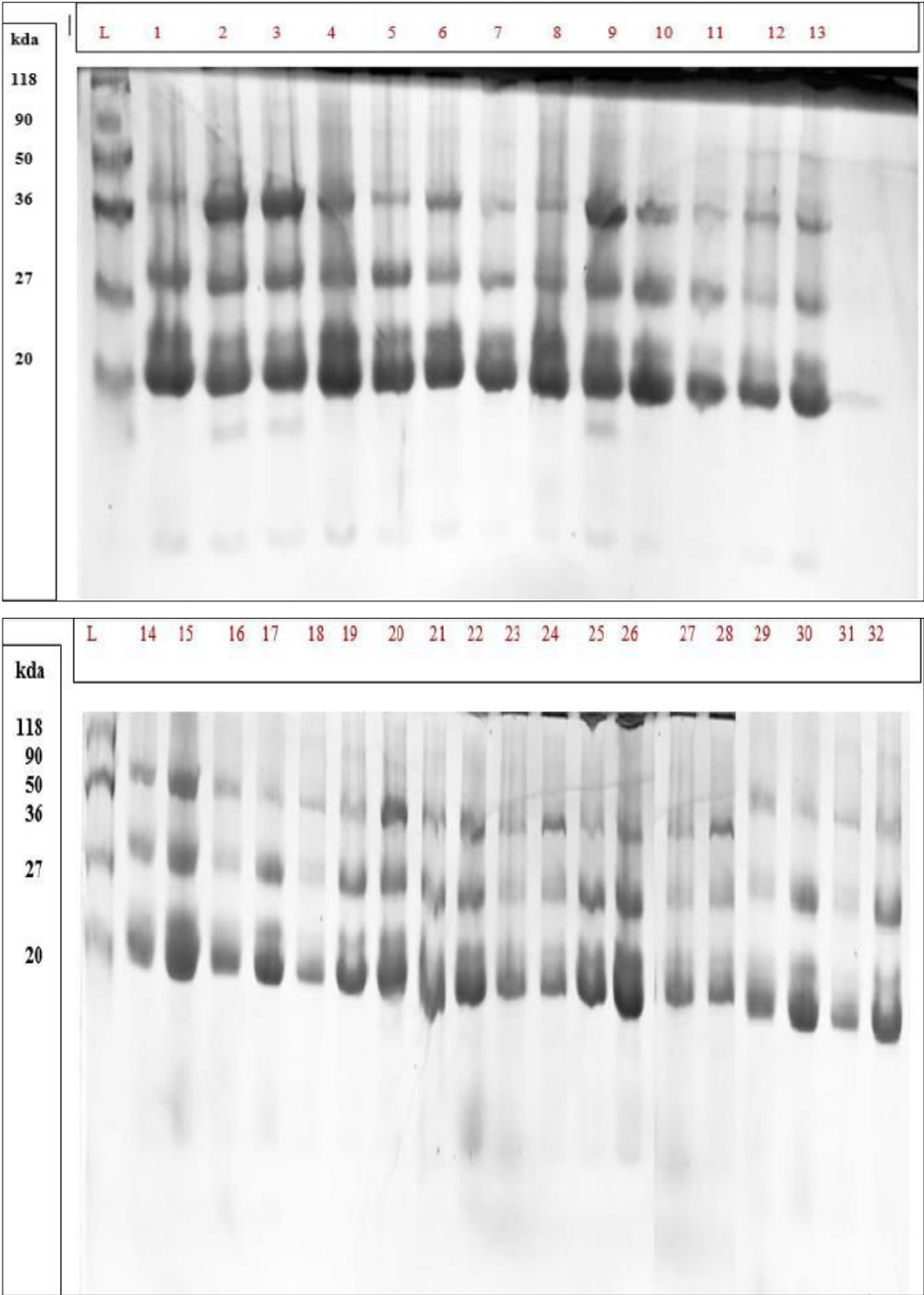


Fig 19: Protein profiling of 32 linseed genotypes under stressed condition

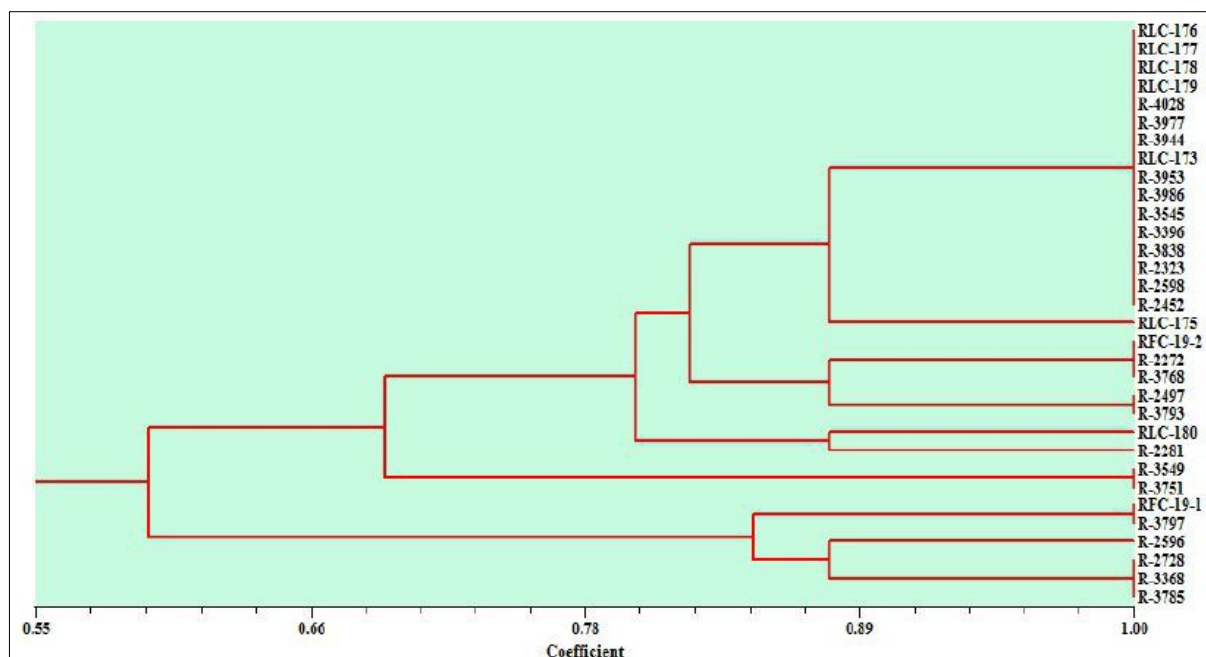


Fig 20: Dendrogram for 32 linseed genotypes under normal conditions based on protein analysis

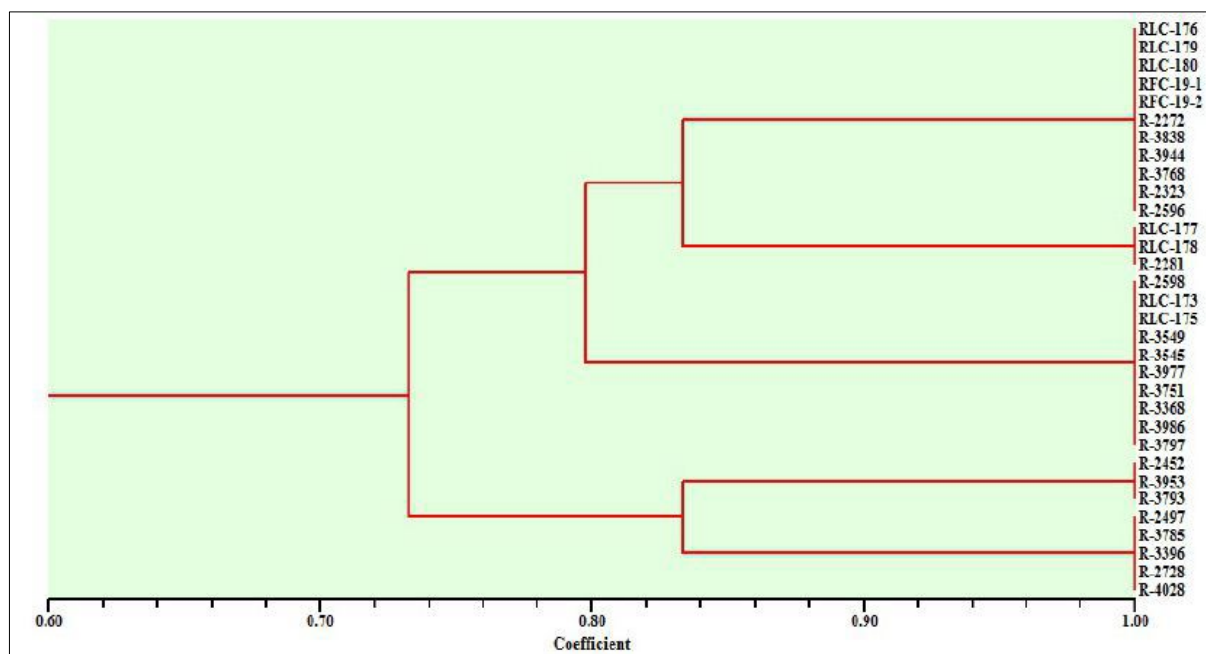


Fig 21: Dendrogram for 32 linseed genotypes under stressed conditions based on protein analysis

Conclusion

The investigation into linseed genotypes, provided a complete understanding of their responses to normal and drought-stressed conditions. The research covered a wide range of morphological and biochemical traits, shedding light on the complex mechanisms of drought tolerance in linseed. The morphological analysis R-3751 exhibited high tolerance, while R-3953 demonstrated susceptibility, particularly in plant height. Seed yield variations were observed, with R-3986 showing flexibility and RLC-177 displaying minimal sensitivity to stress. The highest and lowest 1000 seed weight was recorded in R-176 (9.52 gm) and R-2323 (5.20 gm) respectively under irrigated condition. The highest and lowest 1000 seed weight was recorded in R-3977 (9.13 gm) and R-2497 (4.25 gm) respectively under water stressed condition. The germplasms RLC-176, RLC-179, R-3768, RLC-180, RFC-

19-1, R-3368, and R-3549 shows the similar results for seed length in both stressed and normal condition. No any reduction in seed length was observed in these germplasms. RLC-177 and R-2596 shows the similar seed width under stressed and irrigated condition. Under irrigated condition number of capsules per plant varies from 22 (R-3396) to 170 (R-3986) with an overall average of 79.67. Under stressed conditions range of number of capsules per plant varies from varies from 19 (R-3396) to 114 (R-3986) with average of 53.33. Under stressed conditions the maximum capsule fertility was observed in R-3944 (98.6%). Germplasms R-3785, R-2497, R-2598, R-2728, RFC-19-1, R-2596, R-4028, and R-3977 are susceptible to water stress, exhibiting leaf rolling and decreased leaf relative water content. Each germplasm exhibited varying performance across different morphological traits. Various germplasms exhibited diverse results in biochemical traits such as chlorophyll,

carbohydrate, and proline content. Under water stress, chlorophyll content varies greatly among germplasms, with R-3751 exhibiting the highest content (10.10 mg/g) and R-2598 the lowest (0.74 mg/g). Notably, R-2596 shows minimal decrease (3.89 mg/g) compared to other germplasms under stress. RLC-177, R-2452, R-2323, R-3751, and R-3549 showed increased carbohydrate content, while RFC-19-2, R-2272, R-2281, R-3785, and R-3977 decreased indicating susceptibility of germplasms under stress seed condition. Germplasms RFC-19-1 (11.68 μ mole/gm), RLC-177 (11.09 μ mole/gm), R-2281 (11 μ mole/gm), and R-3368 (10.5 μ mole/gm) showed tolerance to stressed condition by increasing proline content over normal condition. The results from the SDS-PAGE protein profiling revealed the presence of different monomorphic and polymorphic major and minor bands under normal and stressed conditions. Total 8 scorable bands were observed under irrigated condition whereas 6 scorable bands were observed under stressed condition. Under normal conditions, the highest band size of ~60 kDa was present in R-3549 and R-3751. Under stressed conditions, the highest band size of ~35 kDa was present in R-3549 and R-3751. Lowest band size of 1.5 kDa was observed under both condition. Overall, the study suggests a low level of genetic diversity in the total seed protein profiles of the examined in linseed germplasms.

References

- Awari VR, Dalvi US, Lokhande PK, Pawar VY, Mate SN, Naik RM, *et al.* Physiological and biochemical basis for moisture stress tolerance in chickpea under pot study. *Int J Curr Microbiol App Sci.* 2015;6(5):1247-1259.
- Babu K, Rosaiah G. A study on germination and seedling growth of Blackgram (*Vigna mungo* L. Hepper) germplasm against Polyethylene glycol 6000 stress. *IOSR J Pharm Biol Sci.* 2017;12(5):90-98.
- Bates LS, Waldren RA, Teare ID. Rapid determination of free proline for water-stress studies. *Plant Soil.* 1973;39:205-207.
- Biradar SA, Ajithkumar K, Rajanna B, Savitha AS, Shubha GV, Shankergoud I, *et al.* Prospects and challenges in linseed (*Linum usitatissimum* L.) production: A review. *J Oilseed Res.* 2016;33(1):1-13.
- Chaves MM, Maroco JP, Pereira JS. Understanding plant responses to drought from genes to the whole plant. *Funct Plant Biol.* 2003;30(3):239-264.
- Darlington CD. Chromosome botany and the origins of cultivated plants.
- Davatgar N, Neyshabouri M, Sepaskhah AR, Soltani AFShI. Physiological and morphological responses of rice (*Oryza sativa* L.) to varying water stress management strategies.
- De Datta SK, Malabuyoc JA, Aragon EL. A field screening technique for evaluating rice germplasm for drought tolerance during the vegetative stage. *Field Crops Res.* 1988;19(2):123-134.
- Diederichsen A, Ulrich A. Variability in stem fibre content and its association with other characteristics in 1177 flax (*Linum usitatissimum* L.) genebank accessions. *Ind Crops Prod.* 2009;30(1):33-39.
- El-Tayeb MA. Differential response of two *Vicia faba* cultivars to drought: growth, pigments, lipid peroxidation, organic solutes, catalase and peroxidase activity. *Acta Agron Hung.* 2006;54(1):25-37.
- Gamz O, Kaya MD, Atak M. Effects of salt and drought stresses on germination and seedling growth of pea (*Pisum sativum* L.). *Turk J Agric For.* 2005;29(4):237-42.
- Guo R, Hao W, Gong D. Effects of water stress on germination and growth of linseed seedlings (*Linum usitatissimum* L.), photosynthetic efficiency and accumulation of metabolites. *J Agric Sci.* 2012;4(10):253.
- Hernandez T. Influence of soil moisture content at different growth stages on yield of maize in the state of Morelos. *Field Crop Abstr.* 1980;36(3):2240.
- Jayaraman J, Jayaraman J. Laboratory manual in biochemistry. Delhi: Wiley Eastern; c1981. p. 75-76.
- Karamanos AJ. Ways of detecting adaptive responses of cultivated plants to drought: An agronomic approach. *Being Alive on Land;* c1980. p. 13.
- Kariuki LW, Masinde P, Githiri S, Onyango AN. Effect of water stress on growth of three linseed (*Linum usitatissimum* L.) varieties. *SpringerPlus.* 2016;5(1):1-16.
- Kaur V, Yadav R, Wankhede DP. Linseed (*Linum usitatissimum* L.) genetic resources for climate change intervention and its future breeding. *J Appl Nat Sci.* 2017;9(2):1112-1118.
- Kiani R, Nazeri V, Shokrpour M, Hano C. Morphological, physiological, and biochemical impacts of different levels of long-term water deficit stress on *Linum album* Ky. Ex Boiss. Accessions. *Agronomy.* 2020;10(12):1966.
- Khafagy MA, Darowish MM, Salama SM, Abo-El-Kheer ESA. Effect of water priming duration on rice (*Oryza sativa* L.) germination and seedling growth under iso-osmotic solutions of NaCl and PEG. *J Plant Prod.* 2014;5(12):2141-2157.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* 1970;227(5259):680-685.
- Maghsoudi K, Emam Y, Niazi A, Pessarakli M, Arvin MJ. P5CS expression level and proline accumulation in the sensitive and tolerant wheat cultivars under control and drought stress conditions in the presence/absence of silicon and salicylic acid. *J Plant Interact.* 2018;13(1):461-471.
- Merah O. Potential importance of water status traits for durum wheat improvement under Mediterranean conditions. *J Agric Sci.* 2001;137(2):139-145.
- Mohamed SJ. The effect of environmental factors on the physiology, yield and oil composition of safflower (*Carthamus tinctorius* L.) [Doctoral dissertation]. University of Plymouth; 2013.
- Nam NH, Subbarao GV, Chauhan YS, Johansen C. Importance of canopy attributes in determining dry matter accumulation of pigeonpea under contrasting moisture regimes. *Crop Sci.* 1998;38(4):955-961.
- Nayyar H, Gupta D. Differential sensitivity of C3 and C4 plants to water deficit stress: Association with oxidative stress and antioxidants. *Environ Exp Bot.* 2006;58(1-3):1006-1013.
- Pande RC, Singh M, Agrawal SK. Effect of different levels of irrigation, nitrogen and phosphorus on growth,

- yield and quality of linseed (*Linum usitatissimum* Linn.). Indian J Agron. 1970;15:125-130.
27. Panse VG, Sukhatme PV. Statistical methods for agricultural workers. 3rd ed.
 28. Porro I, Cassel DK. Response of maize to tillage and delayed irrigation. Field Crop Abstr. 1986;40(2):637.
 29. Ragupathy R, Rathinavelu R, Cloutier S. Physical mapping and BAC-end sequence analysis provide initial insights into the flax (*Linum usitatissimum* L.) genome. BMC Genomics. 2011;12(1):1-17.
 30. Richharia RH. Linseed. Hyderabad: The Indian Central Oilseeds Committee; c1962. p. 155.
 31. Sammour RH. Proteins of linseed (*Linum usitatissimum* L.), extraction and characterization by electrophoresis. Bot Bull Acad Sin; c1999 .p. 40.
 32. Sardar A, Khan RS, Farooq T, Farook TT. Tissue culture studies in date palm. In: Dates production, processing, food, and medicinal values. p. 13-23.
 33. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother. 2002;56(8):365-379.
 34. Scheller J, Henggeler D, Viviani A, Conrad U. Purification of spider silk-elastin from transgenic plants and application for human chondrocyte proliferation. Transgenic Res. 2004;13:51-57.
 35. Schwenke KD, Dudek S, Mothes R, Raab B, Seifert A. Physico-chemical and enzymatic studies on acetylated protein isolates from faba beans (*Vicia faba* L.). Food/Nahrung. 1993;37(3):258-268.
 36. Tadesse T, Singh HARJI, Weyessa B. Correlation and path coefficient analysis among seed yield traits and oil content in Ethiopian linseed germplasm. Int. J Sustain Crop Prod. 2009;4(4):8-16.
 37. Trevelyan WE, Harrison JS. Studies on yeast metabolism. 1. Fractionation and micro determination of cell carbohydrates. Biochem J. 1952;50(3):298.
 38. Vavilov NI. Studies on the Origin of Cultivated Plants. Institut de Botanique Appliquée et d'Amélioration des Plantes; c1926.
 39. Wakjira A. Linseed (*Linum usitatissimum* L.). In: Vegetable Oils and Fats, Plant Resource of Tropical Africa (PROTA). 2007;14:108-115.
 40. Wang Y, Wang LJ, Li D, Özkan N, Chen XD, Mao ZH. Effect of flaxseed gum addition on rheological properties of native maize starch. J Food Eng. 2008;89(1):87-92.
 41. Zhang ZS, Wang LJ, Li D, Jiao SS, Chen XD, Mao ZH. Ultrasound-assisted extraction of oil from flaxseed. Sep Purif Technol. 2008;62(1):192-198.