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Growth Analysis and Seed Protein Banding Pattern of *Abelmoschus esculentus* L. under Different Water Deficit Durations

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Abstract
Global climate change has resulted in continuous and expanding arid and semi-arid areas and has led to huge losses in crop productivity. Abelmoschus esculentus (okra) is a staple vegetable cultivated and consumed in Sub-Saharan Africa, and thus constitutes a major supply of important nutrients. Therefore, this study was aimed at evaluating and investigating the growth and protein banding patterns of A. esculentus cv. 'Meya' subjected to different durations of drought. The experiment was laid out in a completely randomized design in a screen house with 4 plants per treatment. Vegetative and reproductive data collected were subjected to one-way analysis of variance (ANOVA) and means were separated using Least Significant Difference (LSD). Seed protein profile using SDS-PAGE was also analyzed. Result showed that plants subjected to prolonged drought stress of 10 d and 15 d significantly reduced growth in respect to shoot length, plant height, number of leaves, root length, fresh and dry weights of, shoot and pod but significantly increased the number of pods and dry weight of root. SDS-PAGE analysis showed that control plants expressed more bands in the range of 30 - 50 kDa. In conclusion, results of this study have shown that only plants subjected to longer durations of drought (10 d and 15 d) significantly reduced both vegetative and reproductive growth of A. esculentus cv. 'Meya'. It was also evident in this study that plants subjected to water deficit expressed additional low molecular weight protein bands to enable it cope with the situation.

Keywords*: Abelmoschus esculentus, Growth and productivity, Protein banding, Stress, Water deficit.*

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I. Introduction

Due to global climate change, arid and semi-arid areas in the world have continued to expand. Drought is a major abiotic stress that negatively impacts crop growth and productivity (Alam *et al*., 2020). It reduces biological functions and initiates varying degrees of physiological and biochemical changes in plants (Altaf *et al*., 2015; Jabborova *et al*., 2021). Physiological changes include stomatal closure, decrease in intercellular carbon iv oxide levels and rate of photosynthesis (Chadha *et al*., 2019). More so, due to water deficit, there are significant changes in metabolic processes that occur in photosynthetic components and pigments which alter the enzymatic activities of the Calvin cycle which in turn, affect plant performance (Fu and Huang, 2001; Monakhova and Chernyad'ev, 2002). In drought exposed plants, a hike in biochemical parameters such as reactive oxygen species, superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen leads to cellular damage and death of plant tissues (Alam *et al*., 2020). Other factors of plant growth and development, such as cell enlargement, cell division and cell differentiation are usually impaired owing to drought conditions (Chadha *et al*., 2019). According to Jimoh *et al*. (2018), drought conditions result to very low water content in the soil and makes water uptake difficult for plant roots. Owing to these challenges, complex interactions that involves morphological, physiological, genetic and ecological events are often decreased and prevent plants from attaining its maximum growth potentials (Jaleel *et al*., 2009; Chadha *et al*., 2019). However, reactions of plants to water stress differ significantly and highly depend on intensity and duration of stress as well as plant species and its stage of growth. Therefore, understanding plant responses to drought is of great importance and also a fundamental step for making the crops stress tolerant (Jaleel *et al*., 2009).

Abelmoschus esculentus (okra) is a vegetable crop that belongs to the family Malvacea. It is an annual herbaceous plant indigenous to tropical Africa (Agba *et al*., 2011). They are excellent source of important nutrients and contain appreciable amounts of carbohydrates, energy, protein, dietary fiber, fat, minerals and

vitamins (Adejumo *et al*., 2018). It is grown in most parts of the world including Nigeria because of the pods and leaves which are used as soup thickeners and preparation of stew (Agba *et al*., 2011; Anyaoha *et al*., 2015). Ghana, Burkina Faso and Nigeria are the major producers of Okra in Africa (Jimoh *et al*., 2018). Quite a lot of varieties have been identified and they have been classified based on variations in morphological traits such as fruit size and color, height, earliness and response to photoperiod among other factors (Udoh *et al*., 2005; Anyaoha *et al*., 2015). Despite the boost in agricultural practices and the use of improved and disease-resistant varieties, attaining optimal yield of 2 to 3 tons per hectare is still challenging because of insufficient water supply and climate change (Ghanad *et al*., 2014). Though reports show that okra is relatively drought tolerant (Singh *et al*., 2014), there is still need to monitor its level of tolerance when subjected to varying degrees of water deficit. Altaf *et al*. (2015) noted that the severity of drought can impact yield negatively. Several studies have been reported on the influence of drought on different varieties of okra (Altaf *et al*., 2015; Anyaoha *et al*., 2015; Adejumo *et al*., 2018; Jimoh *et al*., 2018), but none have been reported so far on the staple cultivar 'Meya' which is common in southeastern part of Nigeria. Hence, this research seeks to evaluate the growth responses and seed protein profile of *Abelmoschus esculentus c.v.* 'Meya' under drought-induced stress.

II. Materials and Methods

Study Location

The experiment was conducted in a screen house at the Botanical garden in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, on Latitude 7° 23' East, Longitude 6° 52' North and 447.2 m above sea level (Uguru *et al*., 2012). The study area falls within the derived savannah region of South-Eastern Nigeria. Its annual rainfall ranges from 1500 mm to 1600 mm with an average temperature of 24.9 °C (Eze and Ugwu, 2010; Abu *et al*., 2020).

Collection of Plant Materials /Soil Sample Preparation.

Seeds of *Abelmoschus esculentus* (okra) cv. 'Meya' were purchased from okra seed producers in Adani-Ojo-Ogurugu Agro center, Uzo Uwani LGA Enugu State. Composite top soil and sand obtained from the Botanical Garden were amended with cured poultry manure and mixed in the ratio of 1:2:1 respectively. Mixture was allowed to sit for 7 d before use. Air-dried sample of sterile soil was passed through a 2 mm sieve and analyzed in the Department of Soil Science Laboratory UNN using the standard method of the Association of Official Analytical Chemists (AOAC), 2005 (Table 1).

Seed Viability Test/Growing of Okra Plants.

Test for seed viability was done using the floatation method. All experimental seeds were added into a bowl of water and left for 24 h. Only submerged seeds were selected as being viable. Viable seeds were sown in nursery planting bags and irrigated adequately. Two weeks after sowing (WAS), seedlings were transplanted into medium-sized black polythene bags, measuring 12 cm in diameter and 25 cm deep, filled with sieved soil and arranged in the screen house. Seedlings were allowed to stabilize for 14 days before drought imposition.

Table 1: Physical and chemical properties of soil used for okra growth studies

Parameter	Value
pH (H_2O)	7.100
pH (KCl)	6.200
Sandy soil (%)	64.000
Silt (%)	16.000
Clay (%)	11.000
Organic matter (%)	8.958
Total Nitrogen (%)	1.425
Available P (ppm)	39.104
Exchangeable cations (mg/100 g)	24.641
Calcium (mg/100 g)	8.200
Magnesium (mg/100 g)	17.280
Sodium (mg/100 g)	0.510
Potassium (mg/100 g)	1.450
Hydrogen ion (mg/100g)	1.120

Drought Imposition and Experimental Design

The transplanted seedlings were subjected to four (4) drought conditions as summarized in the table 2. The experiment was laid out in a Completely Randomized Design (CRD) and each treatment was replicated four times.

Table 2: Treatment combinations

Treatment	Abbreviation	Details
Control	Ctrl	Okra plants irrigated every 2 days interval.
Drought (5 d)	D_5	Okra plants subjected to a consecutive 5 days water deficit.
Drought (10 d)	D_{10}	Okra plants subjected to a consecutive 10 days water deficit.
Drought (15 d)	D_{15}	Okra plants subjected to a consecutive 15 days water deficit.

Data Collection of Growth Parameters

Data on plant height; numbers of, leaves, root, pod and flower; area of leaf and root length were collected after 67 days of sowing. Other parameters collected included fresh and dry weights of, shoot, fruit and root.

Protein Extraction and SDS-PAGE

Protein extraction and SDS-PAGE were done according to the methodology of Laemmli, (1970). The seeds used for protein extraction were oven dried and ground into powder using a laboratory mortar and pestle, then the powder was homogenized with an extraction buffer containing 0.05M Tris-HcL pH 7.4 at 4°C, 2.5% sodium dodecyle sulphate (SDS), 10% glycerol and 5% 2-mercaptoethanol. The homogenate was centrifuged at 10,000 g for 15 min at 4°C and supernatant was used for sodium dodecyle sulphate polyacrylamide gel electrophoresis. The cell used consisted of 4% stacking gel and 12% resolving gel. The prepared sample buffer was run on a mini gel apparatus in 5X Tris-Glycine Electrophoresis buffer and Bromophenol blue (BPB) which was as a tracking dye to monitor the movement of protein molecules in the gel. The gels were run at voltage 60 v and gradually through 80 v to 100 v for 2 h in an ominipac mini- vertical gel apparatus using Promega protein as a standard marker. The gel was washed off with 500 ml of the gel-fixing solution and was covered with 400 mL of Coomassie blue stain at room temperature for 3 to 4 h and were gently agitated. Thereafter, the stains were removed by covering the gels with 250 mL of the distaining solution. The distaining solution was changed severally until the protein bands were seen clearly without background staining of the gel. Afterwards, the banding patterns of the treatments were examined for presence and absence of the bands and then photographed.

Statistical Analysis

All data collected were subjected to analysis of variance (ANOVA) using SPSS V.23 and Genstat software. Means and standard deviation were determined at 5% level of probability ($p \leq 0.05$) and the significant means were separated using least significant difference (LSD).

III. Results and Discussion

Findings in this study have shown that plants subjected to 10 d water deficit significantly reduced growth in respect to plant height (PH) and shoot length (SL) (Table 3). Although, not significant, D_5 and D_{15} reduced plant height by 1.68 and 8.82% when compared to control plants, while a significant reduction in plant height (42%) was observed in plants subjected to $D_{10.}$ Shoot lengths of plants in D_5, though not significant, were increased by 6.5% in comparison to control plants. Plants in D_{10} and D_{15} significantly reduced shoot length by 22% and 25% respectively when compared to control plants. Reductions in number of roots (NR), root length (RL), PH, fresh weight of shoot (FWS) and fresh weight of pods (FWP), with prolonged drought, was observed in this study (Table 3 and 4). Significant reductions observed in most of the vegetative and reproductive parameters could be attributed to unfavorable effects of drought stress on plant physiological processes which may have reduced cell division, cell elongation and enlargement or both due to lower relative water content (RWC) and photosynthetic pigments (Tardieu *et al*., 2000; Islam *et al*., 2021). Moreover, previous studies have reported reduction in okra morphological traits owing to water stress (Sankar *et al*., 2008; Kusvuran *et al*. 2008; Kusvuran 2012; Rasmata *et al*., 2014; Adejumo *et al*., 2018; Mueller *et al*., 2019). In line with this study, Zhao *et al*. (2006) and Raza *et al*. (2012), also reported decreased plant height due to drought stress in *Brassica napus* and wheat. More so, drought stress has significantly decreased the germination rate and plant growth in various crops (Hussain *et al*., 2016; Egamberdieva *et al*., 2017; Jabborova *et al*., 2020; Sheteiwy *et al*., 2021). Drought conditions had also been observed to limit the uptake of nutrients by the plants due to limited soil moisture, leading to decreased stem length (Razmjoo *et al*., 2008; Gheidary *et al*., 2017). This also may have possibly resulted to reduced shoot length in plants subjected to prolonged stress as was observed in this study. This finding also corroborated the reports of Gheidary *et al*. (2017) who reported that shoot length was reduced under water deficit conditions in *Lathyrus sativus* L. According to Bhatt and Srinivasa, (2005), leaf wilting is one of the visible indicators of water stressed plants. In this study, though not significant, plants in D_5 increased the number of leaves by 11.11%, while plants in D_{10} and D_{15} significantly reduced the number of leaves by 11.11% and 40.75% respectively. In an interesting manner the number of leaves significantly reduced among drought regimes following this yielded pattern, that is $D_{15} > D_{10} > D_{5.}$ This result is in concord with the study of Abbas (2007) who also reported a greater number of leaves in okra when irrigation was applied at 8 days interval compared to 16 and 24-days interval. Though not significant, drought exposed okra plants in this study yielded

wider leaves when compared to the control plants (Table 3). This finding disagrees with the reports of other researchers who reported a reduced leaf area in okra plants due to drought stress (Altaf *et al*., 2015; Adejumo *et al*., 2018). A drought imposition of 10 d (D_{10}) significantly reduced root length by 50.85% when compared to that recorded in control plants. However, other drought regimes did not significantly reduce the length of roots. The reduction of root length with prolonged drought as observed in this study could be attributed to decreases in cell division and differentiation because of inadequate water provisions (Tardieu *et al*., 2000).

Though, not significant, okra plants grown under prolonged drought stress yielded less flowers compared to the control plants except for plants subjected to 5 d drought stress (Table 3). This finding is in line with the reports of Anyaoha *et al*. (2015), which asserted that moderate stress conditions in okra only supported minimal development of flowers while extreme stress conditions did not support any flower development. This report disagrees with the belief that plants possess the ability to produce more flowers under stressed conditions (Alam *et al*., 2020). Okra plants exposed to prolonged drought for 15 d (D_{15}) yielded the highest number of pods as compared to that in the control (Table 3). Number of pods from plants in other drought regimes (D_5 and D_{10}) did not differ significantly from that in control plants. Moreover, okra fruit yield was generally enhanced under water deficit than the control plants and this could be attributed to okra's ability to survive and tolerate water deficit with the help of different osmolytes synthesized (Abid *et al*., 2002; Verbruggen and Hermans, 2008), all of which might have contributed to greater adaptation and performance than control plants. This report corroborates with the earlier documentations of Jimoh *et al*. (2018) in okra and Yaza *et al*. (2002) in tomato and cotton. Reduced root length with prolonged drought was observed in this study (Table 3). This could be due to decreases in cell division and differentiation because of inadequate water provisions (Tardieu *et al*., 2000). Studies have attributed the reduction in okra morphological traits due to drought stress (Sankar *et al.,* 2008; Kusvuran *et al*. 2008; Rasmata *et al*., 2014; Adejumo *et al*., 2018).

Influence of drought stress on biomass of okra revealed that plants subjected to 10 and 15 d water deficit significantly reduced the fresh weights of shoots by 64% and 56% respectively when compared to control plants, while D_5 also reduced fresh weight of shoot, although not significantly different from that in control plants (Table 4). More so, significant reduction on fresh weight of pod was also observed, with plants in D_5, D_{10} and D_{15} having significantly lower fresh weights when compared to control plants. However, there was no significant variation recorded on the fresh weight of the roots. Significant reductions recorded in fresh weight of shoot and pods of drought stressed plants, in this study, is an indicator of the negative effects of decreased photosynthetic pigments that might have impaired the rate of photosynthesis and dry matter production (Islam *et al*., 2021). This report agrees with the findings of Altaf *et al*. (2015), which recorded significant reductions in the fresh shoot weight of okra plants grown under drought conditions. Though not significant, dry weight of okra shoot and pods were reduced at different durations of drought stress when compared to control plants. This finding disagrees with studies of Altaf *et al*. (2015) and Chadha *et al*. (2019) which noted that total fresh and dry weights of *Abelmoschus esculentus* and *Lactuca serriola* were significantly reduced with drier soil moisture regimes.

Evaluation of seed protein profiling via SDS-PAGE for all treatments showed several different banding patterns. Control plants expressed a total of 4 bands (Figs 1 and 2). Interestingly, plants subjected to D_5 and D_{15} expressed lower molecular weight proteins as compared to the control, which could be attributed to the possible damaging effect of drought stress (Table 5). Moreover, this observation could be an indication of the capability of okra plants to produce new low molecular weight proteins when subjected to drought stress. This finding agrees with several studies that showed the expression of low molecular weight proteins being produced in plants undergoing abiotic stress (Duressa *et al*., 2010; Egedigwe, 2012). Though the mechanism of infection/ resistance proteins in plant are poorly understood, Egedigwe, (2012) opined that the production of some new proteins may likely be responsible for plants developing resistance under direct water stress.

IV. Conclusion

This study has shown that the ability of *A. esculentus* to withstand water deficit is in its capacity to shade leaves and increase leave area in order to enhance photosynthesis. Okra exposed to prolonged water stress was observed to yield a lower productivity which could be a serious limiting factor in the growth and development of okra. The SDS-PAGE analysis showed the expression of low molecular weight protein bands which could be attributed to the possible effects of drought in the stressed plants. Though not included in this study, going down to the molecular mechanisms as well as studying the specific proteins responsible for drought conditions will be germane to engineering drought resistant crops which will be suitable for conditions seen in Sub- Saharan Africa.

Table 3: Vegetative and reproductive attributes of *Abelmoschus esculentus* under different durations of water deficit.

Treatments	Number of roots	Shoot length (cm)	Plant Height (cm)	Root Length (cm)	Number of Leaves	Leaf Area (cm^2)	Number of Flowers	Number of Pods
D_5	9.25 ± 0.85	33.25± 1.11[a]	58.50 ± 7.10[a]	26.50 ± 1.26[a]	7.50 ± 0.29[a]	284.6± 15.34	5.50 ± 1.19	3.25 ± 0.25[ab]
D_{10}	9.75 ± 2.29	22.25± 1.65[b]	34.50 ± 3.23[b]	14.25 ± 1.03[b]	6.00 ± 0.41[b]	150.0± 7.10	4.50 ± 0.65	2.50 ± 0.29[b]
D_{15}	8.75 ± 0.85	24.50± 0.96[b]	54.25 ± 8.67[ab]	28.50 ± 0.65[a]	4.00 ± 0.00[c]	205.8± 30.42	3.50 ± 0.29	4.00 ± 0.41[a]
Control	11.50 ± 1.32	31.25± 1.11[a]	59.50 ± 5.33[a]	29.00 ± 1.47[a]	6.75 ± 0.48[ab]	146.3± 22.1	4.75 ± 0.63	2.50 ± 0.29[b]
LSD	NS	4.40	22.82	4.07	1.23	NS	NS	1.12

Means sharing similar letters in a group are statistically not significant at ($p \leq 0.05$). NS = Not significant.

Table 4: Effect of drought stress on fresh weight of shoots, roots and pods of *A. esculentus*

Treatments	Fresh Weight of Shoot (g)	Fresh Weight of Root (g)	Fresh Weight of Pods (g)	Dry Weight of Shoot	Dry Weight of Root	Dry Weight of Pods
D_5	27.85 ± 2.56[a]	11.30 ± 1.05	19.68 ± 2.03[b]	2.78 ± 0.83	1.33 ± 0.06[a]	5.33 ± 1.91
D_{10}	10.63 ± 0.47[b]	10.83 ± 0.55	16.00 ± 2.11[c]	2.50 ± 0.60	0.43 ± 0.33[bc]	3.88 ± 1.66
D_{15}	12.78 ± 1.98[b]	11.85 ± 1.04	20.13 ± 0.60[b]	3.83 ± 0.69	1.18 ± 0.17[ab]	2.75 ± 0.88
Control	29.33 ± 7.15[a]	11.00 ± 0.66	26.35 ± 2.32[a]	3.05 ± 1.66	0.39 ± 0.27[c]	6.90 ± 2.60
LSD	13.90	NS	2.73	NS	0.82	NS

Means sharing similar letters in a group are statistically not significant at ($p \leq 0.05$). NS = Not significant.

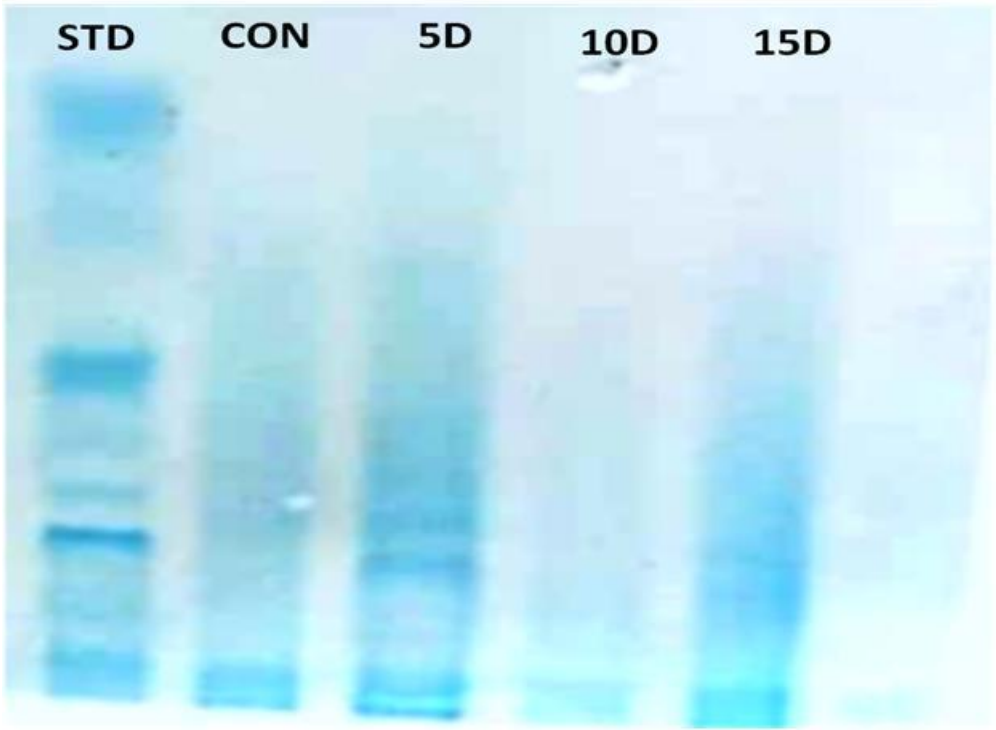

Fig. 1: Protein gel showing banding pattern of *A. esculentus* plant under different drought durations. Std- standard; con- control, 5D- 5days of drought; 10D-10days of drought; 15D -15days of drought.

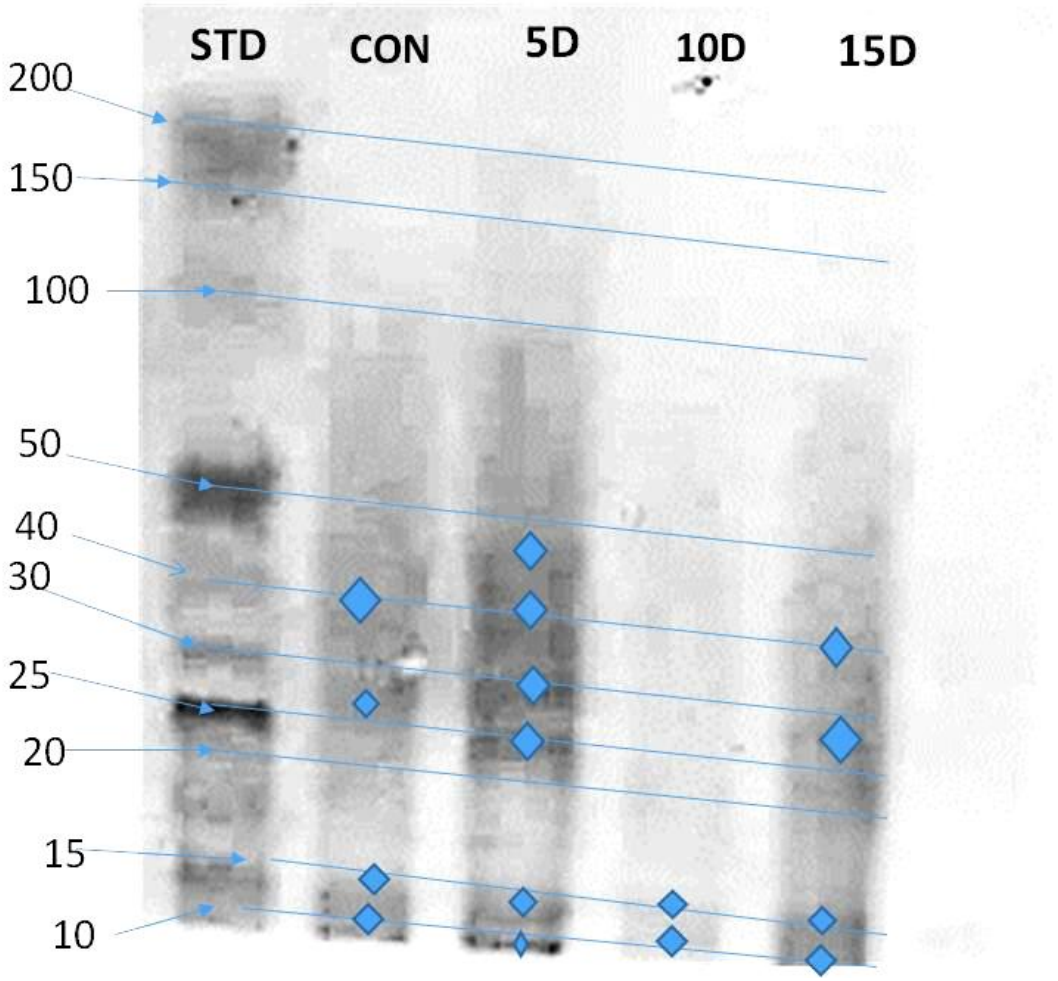

Fig. 2: Analyzed protein gel showing banding patterns of both drought stressed and control plants; Std-standard; con- control; 5D-5days of drought; 10D-10days of drought; 15D -15days of drought.

Table 5: Relative molecular weights of the analyzed samples

Bands	Con	D5	D10	D15
1	40	48	15	43
2	26	40	10	28
3	15	30		23
4	10	25		15
5		15		10
6		10		

Con – control; D5- 5d drought stress; D10- 10d drought stress; D15- 15d drought stress.

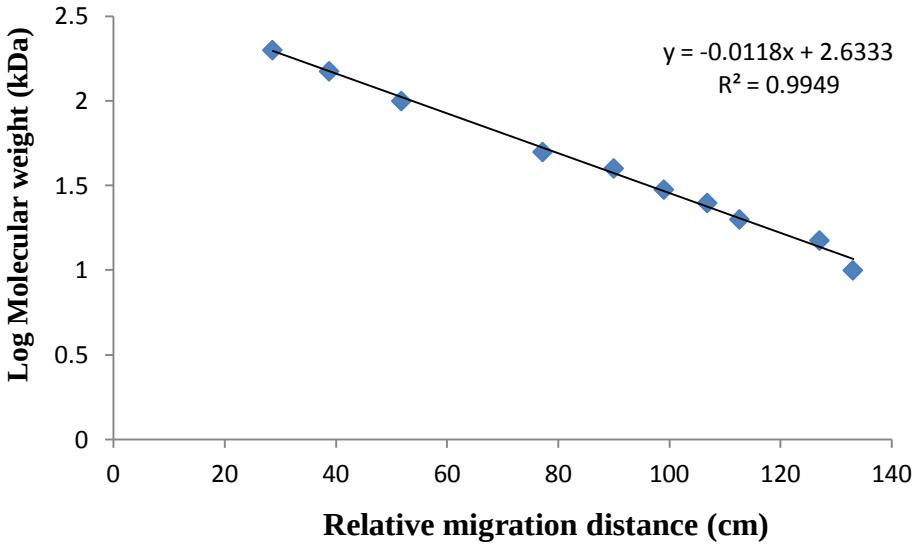

Fig. 3: Standard curve for the determination of molecular weights of treatments bands

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