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CHARACTERISATION OF DROUGHT-INDUCED ALTERATIONS IN SEED PROTEIN CHARACTERISTICS OF *Cicer arietinum* L.

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ABSTRACT

To study the impact of drought stress on seed protein quantity and quality of *Cicer arietinum* (chickpea), different chickpea cultivars, including the drought-tolerant (HC5) and drought-sensitive (HC1), were subjected to varying levels of drought stress (Control, L1- 10% soil moisture, L2- 5% soil moisture). The total seed protein content increased in both genotypes under drought stress, with a comparatively higher increase (2.7%) in the drought-tolerant genotype. In the HC5 genotype, the proportions of albumins and prolamins decreased, while globulins and glutelins increased under reduced soil moisture conditions. The drought-sensitive genotype showed a similar trend, although the changes were less pronounced. Amino acid analysis revealed variations in tryptophan, methionine, and cysteine content in different protein fractions isolated from seeds of drought-stressed plants. The SDS gel electrophoretic analysis of the four seed protein fractions primarily revealed quantitative alterations in the polypeptides with minor qualitative alterations under drought stress. Thus, our study revealed that drought stress impacts seed protein quality in chickpea by affecting the proportion of four protein fractions and the content of sulphur-containing amino acids.

Keywords: Amino acids, *Cicer arietinum*, Drought, SDS-gel electrophoresis, Seed proteins

The State of Food Security and Nutrition in the World (SOFI, 2021) reported that nearly one-tenth of the world's population i.e. 811 million people went hungry in 2020, with many not getting the required proteins and being malnourished. The hunger level has reached an unprecedented level, with nearly 193 million undernourished people facing acute food insecurity worldwide, a significant increase from the previous year (GRFC, 2022). The COVID-19 pandemic has also exacerbated the problem, as many people have run out of food or reduced their consumption, leading to compromised nutrition and protein deficiency (World Bank, 2020). Furthermore, projections suggest that by 2030, approximately 660 million individuals could potentially experience hunger, which is 30 million higher than the anticipated number in a scenario unaffected by the pandemic (SOFI, 2021). It is now widely acknowledged that although some progress has been made in tackling malnutrition and protein deficiency, the world still needs to catch up in achieving its global nutrition targets by 2030 (Development Initiatives, 2020; UNICEF, 2020). Consequently, it is crucial to implement comprehensive and immediate measures to mitigate the adverse effects of the pandemic and attain the global objectives of combating hunger, malnutrition, and micronutrient deficiencies by 2030. Incorporating legumes into the diet could be a potential strategy to address the situation, particularly for poor and vegetarian individuals (Ferreira *et al.*, 2021).

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Among legumes, chickpea plays a significant role as a valuable protein source for vegetarians (Kudapa *et al*., 2013). It contains 16-28% seed proteins, 41% carbohydrates, and 3-6% oil and essential minerals such as potassium, iron, calcium, manganese, phosphorus, magnesium, and zinc (Jukanti *et al*., 2012). The seed proteins are constituted mainly of globulins (70%) and albumins (20%), and glutelins and prolamins constitute protein fractions in relatively small proportion (Duranti, 2006). The balance of essential and non-essential amino acids in chickpea seed proteins is favourable for human health and nutrition (Jukanti *et al*., 2012). Moreover, consuming chickpea seeds has been associated with several health benefits, including hypocholesterolemic effects (Faridy *et al*., 2020), the mitigation of the severity of type 2 diabetes (Li *et al*., 2021), and a reduced risk of certain types of cancer, such as colorectal cancer (Cummings *et al*., 1981; Higdon and Frei, 2003) and stomach cancer (Dixon, 2004).

However, chickpea productivity and yield are negatively affected by various abiotic stresses such as drought, heat and salinity, as well as biotic stresses such as fungal diseases (Jha *et al*., 2014). These stresses reduce chickpea growth, yield and quality and thus limit its potential to serve as a source of dietary proteins. Drought considerably affects crop growth and productivity among abiotic stresses, with profound implications on food security. The negative impacts of abiotic stresses on seed protein content and quality can significantly affect people's health and nutrition globally. The WHO has estimated that nearly 2 billion people

worldwide suffer from protein deficiency, which can lead to various health problems (WHO, 2007).

Furthermore, climate change is expected to aggravate the problem of protein deficiency as abiotic stresses become more frequent and severe, leading to reduced crop yields and lower seed protein content (Chaudhry and Sidhu, 2022). Recent studies have shown that changes in rainfall patterns and temperature can lead to drought stress in plants (Lobell *et al*., 2013). Also, salinity stress (Munns and Tester, 2008), poor soil aeration (Bengough *et al*., 2011), and elevated levels of atmospheric CO_2 (Morgan *et al*., 2011) can contribute to drought stress. The proportion of four seed protein fractions, namely, albumins, globulins, glutelins and prolamins is affected by drought stress as reported in various crops, such as common bean (Ghanbari *et al*., 2015), soybean (Zhou *et al*., 2022), maize (Ortiz-Martinez *et al*., 2017), wheat (Rekowski *et al*., 2021), lentil (Sehgal *et al*., 2018), faba bean (Gasim *et al*., 2015) etc. However, drought's effect on chickpea seed protein fractions is yet to be studied. Therefore, given the significance of chickpeas in human nutrition and the increasing prevalence of drought in broad regions, this study was undertaken to investigate the effects of drought stress on seed protein fractions in chickpeas.

MATERIALS AND METHODS

The seeds of chickpea genotype HC5 (drought-tolerant) and HC1 (drought-sensitive) used in the present study were obtained from Chaudhary Charan Singh Haryana Agricultural University (CCS HAU) located in Hisar, Haryana, India. A pot experiment was conducted during the winters of year 2022 to investigate effect of drought stress on seed protein fractions. The experiment was conducted in the net-house of Department of Botany, Kurukshetra University, Kurukshetra, India. The soil-filled pots were divided into three sets comprising two sets for drought treatments and one set as a control, each with five replicates. Additionally, thinning was carried out to maintain five plants per pot. Before the onset of flowering, the water supply was controlled to generate and maintain moisture content (drought levels) to 10% (L1) and 5% (L2) compared to 30% moisture content in control plants by given formula**.**

These levels were maintained till seed maturity and after seed harvesting, the seeds were dried and subsequently ground to seed meal. Furthermore, the seed meal was defatted for various seed protein characteristics analyses.

Total seed protein estimation

The semi-micro Kjeldahl method, devised by Peach and Tracey (1956) was used to quantify the total nitrogen in 100mg of seed meal. The calculated nitrogen percentage was multiplied by 6.25, a standard multiplication factor to determine the total seed protein content.

Fractionation of seed proteins

The method described by Croy *et al.* (1984), with little modification,was employed for seed protein fractionation. Specifically, albumins and globulins were extracted in 50 mM borate buffer at pH 8, followed by dialysis to separate each fraction. Glutelins and prolamins, on the other hand were isolated using 0.1N NaOH and 70% ethanol, respectively.

The proportion of four protein fractions

The four seed protein fractions were quantified using Bradford's method (Bradford, 1976). Subsequently, the final protein concentration in each fraction was determined by referencing a standard curve constructed using bovine serum albumin (BSA).

SDS-polyacrylamide gel electrophoresis

The polypeptide pattern of the four seed protein fractions was studied by electrophoretic analysis on SDS gels, following the discontinuous system developed by Davis (1964) and Ornstein (1964) and the methodology proposed by Laemmli (1970). The gels were quantitatively analysed by using GelAnalyzer version 19.1. To simplify our data and graphs, we divided all collected raw values by thousand in our computations.

Tryptophan, Methionine and Cysteine Estimation

Tryptophan content was estimated using the methodology outlined by Spies and Chambers (1948). Methionine was estimated by following the protocol prescribed by McCarthy and Sullivan (1941) and the analytical approach devised by Goa (1961) was used for the estimation of cysteine content.

Statistical analysis

The data presented in the tables and figures are represented as the mean value ± standard error (SE) as a measure of variability. A mean of 3 readings was taken in each replicate. Statistical analysis was done using Microsoft Excel version 2010 and Statistical Packages for Social Sciences (SPSS) version 16.0. A post-hoc test (Duncan) using the same software was used to determine the difference among data. One-way ANOVA was employed to assess statistically significant differences among the various estimations.

RESULTS AND DISCUSSION

Total seed protein content and four seed protein fractions

Fig. 1 illustrates the variation in seed protein content across different levels of drought. With a decrease in moisture content to 5% (L2), the protein content showed an increase from 25.1% in control to 27.8% in drought-tolerant genotype (HC5) and from 24.1% to 25.2% drought-sensitive (HC1). The increase in protein content was higher in the tolerant genotype (2.7%). A moderate drought during flowering can cause a 6–21% increase in grain protein content (Behboudian *et al*., 2001). The increase in seed proteins under drought stress has also been reported earlier in many crops like a common bean (Kazai *et al*., 2019), soybean (Mertz-Henning *et al*., 2017), mung bean (Al-Suhaibani, 2009), chickpea (Behboudian *et al*., 2001; Samineni *et al*., 2022), spotted bean (Ardakani *et al*., 2013), wheat (Gooding *et al*., 2003), potato (Teixeira and Pereira, 2007) and rapeseed (Bouchereau *et al*., 1996). Under drought stress conditions, a plausible explanation could be the upregulation of genes encoding stress-responsive proteins (Takahashi *et al*., 2018; Todaka *et al*., 2019). Increased protein content might be due to a change in C/N ratio, an outcome of altered C-partitioning, ultimately favouring more N-assimilation (Sehgal *et al*., 2018). Also, the expression of proteins which are involved in various biological processes such as photosynthesis, antioxidant enzymes, signal transduction, ATP synthase and other nitrogen metabolism-related proteins, along with heat shock proteins (HSPs) were enhanced in the tolerant genotype compared to the sensitive one (Wang *et al*., 2015). During the initial stages of seed filling under unfavourable conditions, it is likely that proteins responsible for plant protection under stress are also synthesised, leading to an increase in seed protein content. (Khazaei *et al*., 2019).

The effect of different levels of drought stress on four protein fractions can be seen in Fig. 2. Changes in protein fractions under drought stress have also been reported in wheat (Zhang *et al*., 2014) and rice (Wang *et al*., 2003). In genotype HC5, with the decrease in moisture content from L1 (10%) to L2 (5%), the albumins decreased from 31.8 mg/g to 22.7 mg/g seed meal. The globulins increased from 130.7 mg/g to 137.8 mg/g of seed meal. Glutelins followed the increasing trend, while a decreasing trend was observed in the prolamins. On the other hand, in the HC1 genotype with an increase in drought levels, albumins decreased from 29.8 mg/g to 23.1 mg/g, prolamins from 5.4 mg/g to 3.8 mg/g, while globulins increased from 125.8 mg/g to 132.1 mg/g and glutelins increased from 25.4 mg/g to 29.3 mg/g seed meal.

The relative proportion of globulins and glutelins grew more (66.6% to 67.1% and 13.4% to 14.5%, respectively), while that of albumins and prolamins decreased in the case of the drought-tolerant genotype (14.7% to 13.6% and 5.3% to 4.8%, respectively) (Table 1). The decrease in the proportion of albumins and prolamins (from 14.9% to 14.0% and 5.1% to 4.3%, respectively) was more in the drought-tolerant than drought-sensitive genotype. On the other hand, there was an increase in the levels of glutelins and globulins, which rose from 14.5% to 15.3% and 65.5% to 66.4%, respectively (Table1). Our results are in agreement with the findings of Alghamdi (2009), in which a fall in the level of seed albumins and prolamins, along with globulins, was observed in faba beans grown under drought stress. On the other hand, glutelin concentrations increased, and overall protein content increased. In the context of wheat, it was observed that the concentrations of albumin and gliadin exhibited a marked increase in response to drought conditions, as documented by Zhang *et al*. (2014). These findings suggest that drought-tolerant genotypes may have a higher capacity to modulate seed protein fractions as a result of drought-induced stress, potentially improving the nutritional quality of the seed under stress conditions (Zhang *et al*., 2014; Mousavi-Derazmahalleh *et al*.,

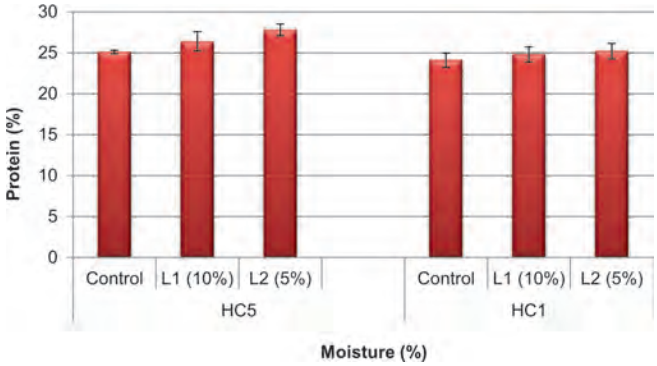

Fig. 1. The seed protein content (%) under different drought stress levels

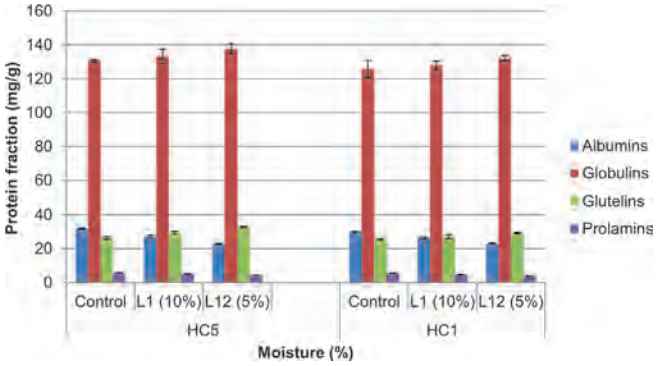

Fig. 2. Effects of drought stress on four seed protein fractions

Table 1. Effect of drought stress on the proportion of four protein fractions

Genotype	Moisture (%)	Proportion of four fractions (%)			
		Seed protein fractions			
		Albumins	Globulins	Glutelins	Prolamins
HC5	Control	14.7 ± 0.09^{ab}	66.6 ± 1.19^{a}	13.4 ± 0.26^{d}	5.3 ± 0.09^{a}
	L1 (10%)	14 ± 0.15^{cd}	66.8 ± 0.89^{a}	14.1 ± 0.18^{c}	5.1 ± 0.12^{ab}
	L2 (5%)	13.6 ± 0.15^{d}	67.1 ± 0.26^{a}	14.5 ± 0.12^{bc}	4.8 ± 0.06^{c}
HC1	Control	14.9 ± 0.21^{a}	65.5 ± 0.54^{a}	14.5 ± 0.09^{bc}	5.1 ± 0.06^{abc}
	L1 (10%)	14.4 ± 0.06^{bc}	65.8 ± 0.83^{a}	14.9 ± 0.36^{ab}	4.9 ± 0.12^{bc}
	L2 (5%)	14 ± 0.09^{cd}	66.4 ± 1.49^{a}	15.3 ± 0.15^{a}	4.3 ± 0.03^{d}

Each value represents the mean of three replicates ± SE
In a column, values followed by same alphabet do not differ significantly at p≤0.05

2019). Drought tolerant genotype shows an upsurge in the proline level as a response to exposure to moderate level of drought, ten times higher than normal, at the vegetative stage (Anjum *et al*., 2011). This drought-induced solute accumulation has boosted drought resistance in chickpeas (Amede *et al*., 2003). This enhanced proline accumulation help in osmotic adjustment that improves the plant's performance (Mafakheri *et al*., 2010).

Amino acids

The content of three amino acids, tryptophan, methionine, and cysteine, was assessed in the four protein fractions separated from the seeds of plants raised under different drought levels in both genotypes (Table 2-4). Exposure of the HC5 genotype to higher levels of drought stress led to a decline in total tryptophan content due to globulins and prolamins, while an increase due to a rise in albumins and glutelins content with the reduction of soil moisture content was observed (Table 2). The sensitive genotype with increasing levels of drought exhibited an increase in globulins and prolamins and decrease in albumins and glutelins (Table 2). Gargallo-Garriga *et al*. (2018) found that the tryptophan content increased in drought-tolerant soybean genotypes under drought stress. Similarly, Rao *et al*. (2012) and Khan *et al*. (2019) observed an increase in tryptophan content in maize leaves and chickpea genotypes under drought stress, respectively.

The total methionine in the minor fractions (prolamins and glutelins) followed an increasing pattern in the tolerant genotype, while major fractions (albumins and globulins) pursued a decreasing pattern (Table 3). In the drought-sensitive genotype, total methionine by glutelins increased under increasing drought levels. In contrast, methionine contribution by albumins, globulins and prolamins experienced a drop in values, as shown in Table 3. Methionine content decreased due to albumins and globulins in both genotypes, but the extent of the loss was higher in the sensitive genotype. Mohammadkhani and Heidari (2008) found that methionine content increased under the influence of drought in drought-tolerant maize genotypes. Girija *et al*. (2020) reported a positive correlation between

Table 2. Effect of drought stress on total tryptophan content of four protein fractions in drought-tolerant and drought-sensitive lines

Genotype	Moisture (%)	Total tryptophan content (g/100g protein)			
		Fractions			
		Albumins	Globulins	Glutelins	Prolamins
HC5	Control	1.12 ± 0.03^{d}	3.85 ± 0.01^{a}	1.03 ± 0.00^{c}	0.64 ± 0.01^{a}
	L1 (10%)	1.17 ± 0.02^{cd}	3.7 ± 0.03^{b}	1.09 ± 0.02^{b}	0.61 ± 0.01^{b}
	L2 (5%)	1.23 ± 0.03^{bc}	3.55 ± 0.05^{c}	1.14 ± 0.02^{b}	0.57 ± 0.01^{c}
HC1	Control	1.4 ± 0.02^{a}	3.24 ± 0.02^{d}	1.23 ± 0.01^{a}	0.38 ± 0.01^{f}
	L1 (10%)	1.29 ± 0.02^{b}	3.34 ± 0.01^{d}	1.16 ± 0.01^{b}	0.44 ± 0.01^{e}
	L2 (5%)	1.18 ± 0.01^{cd}	3.5 ± 0.04^{c}	1.02 ± 0.01^{c}	0.51 ± 0.01^{d}

Each value represents the mean of three replicates ± SE
In a column, values followed by same alphabet do not differ significantly at p≤0.05

Table 3. Effect of drought stress on total methionine content of four protein fractions in drought-tolerant and drought-sensitive lines

Genotype	Moisture (%)	Total methionine content (g/100g protein)			
		Seed protein fractions			
		Albumins	Globulins	Glutelins	Prolamins
HC5	Control	1.45 ± 0.04^{c}	3.06 ± 0.01^{a}	1.1 ± 0.02^{e}	0.34 ± 0.01^{e}
	L1 (10%)	1.38 ± 0.02^{c}	2.99 ± 0.02^{a}	1.15 ± 0.02^{de}	0.36 ± 0.01^{e}
	L2 (5%)	1.3 ± 0.01^{d}	2.89 ± 0.02^{b}	1.21 ± 0.01^{d}	0.4 ± 0.01^{d}
HC1	Control	1.71 ± 0.04^{a}	2.83 ± 0.02^{b}	1.31 ± 0.02^{c}	0.74 ± 0.02^{a}
	L1 (10%)	1.55 ± 0.02^{b}	2.68 ± 0.05^{c}	1.39 ± 0.02^{b}	0.67 ± 0.01^{b}
	L2 (5%)	1.46 ± 0.02^{c}	2.55 ± 0.03^{d}	1.53 ± 0.04^{a}	0.59 ± 0.01^{c}

Each value represents the mean of three replicates ± SE
In a column, values followed by same alphabet do not differ significantly at p≤0.05

methionine content and glutelins and prolamins in *Arabidopsis*, while albumins and globulins showed a negative correlation.

Total cysteine increased due to albumins, glutelins and prolamins in the drought-tolerant genotype but decreased due to the globulin fraction (Table 4). However, in the drought-sensitive genotype, cysteine content increased due to albumins and globulins but decreased due to glutelin and prolamin fractions, respectively, under drought stress. Our results are aligned with the findings of Nuccio *et al*. (2015) in wheat, in which total cysteine content increased in the drought-tolerant genotype under drought stress but decreased in the drought-sensitive genotype. Additionally, Marcolino-Gomes *et al*. (2015) found that the response of cysteine content to drought stress in soybean leaves varied among cultivars. All of these studies suggest that the response of amino acid content to drought stress varies among plant species and genotypes, and that certain amino acids may confer drought tolerance (Khan *et al*., 2020; Chapman *et al*., 2022).

Polypeptide patterns on SDS-gels

Electrophoretic analyses of total proteins and four protein fractions, extracted from seeds of plants cultivated in soil with varying levels of moisture content, were carried out on SDS gels under reducing conditions (Fig. 3.2-3.3). In addition, standard protein molecular weight markers were also run along with the samples on the gel (Fig. 3.1) to determine the molecular weights of polypeptides.

In the genotype HC5 (Fig. 3.2), the polypeptides in the total seed protein extract varied in their molecular weight from 20 kDa to 108 kDa. Variations in intensities of polypeptides of four fractions in drought tolerant genotype can be seen in Fig. 4.4, 5.4 and 6.4. The globulins from optimally irrigated (control) plants were composed of many polypeptides ranging in their molecular weights from *Mr* 14 kDa to 65 kDa. The polypeptide of *Mr* 24 kDa was found to be intense and dark with 2.02 band intensity (Fig. 5.1-5.3), while *Mr* 32 kDa and 26 kDa polypeptides were noticeable but of relatively low intensity (1.06 and 1.49, respectively).

Table 4. Effect of drought stress on total cysteine content of four protein fractions in drought-tolerant and drought-sensitive lines

Genotype	Moisture (%)	Total cysteine content (g/100g protein)			
		Seed protein fractions			
		Albumins	Globulins	Glutelins	Prolamins
HC5	Control	0.9 ± 0.02^{a}	1.89 ± 0.01^{a}	0.64 ± 0.00^{d}	0.22 ± 0.01^{c}
	L1 (10%)	0.87 ± 0.01^{a}	1.84 ± 0.03^{ab}	0.69 ± 0.01^{c}	0.24 ± 0.01^{c}
	L2 (5%)	0.81 ± 0.02^{b}	1.76 ± 0.02^{b}	0.75 ± 0.02^{b}	0.26 ± 0.01^{b}
HC1	Control	0.54 ± 0.01^{d}	1.56 ± 0.04^{c}	0.82 ± 0.02^{a}	0.31 ± 0.01^{a}
	L1 (10%)	0.59 ± 0.02^{c}	1.61 ± 0.04^{c}	0.76 ± 0.02^{b}	0.3 ± 0.01^{a}
	L2 (5%)	0.63 ± 0.02^{c}	1.65 ± 0.04^{c}	0.72 ± 0.02^{bc}	0.26 ± 0.01^{b}

Each value represents the mean of three replicates ± SE
In a column, values followed by same alphabet do not differ significantly at p≤0.05

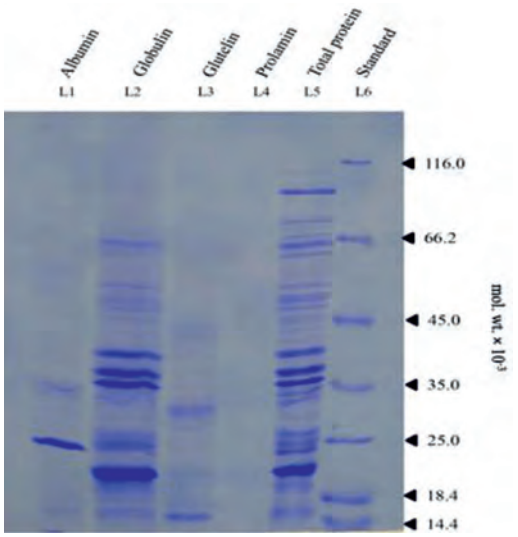

Fig. 3.1. SDS-PAGE of seed protein fractions, total seed proteins in chickpea and standard protein markers
L1: Albumins, L2: Globulins, L3: Glutelins, L4: Prolamins, L5: Total proteins, L6: Standard protein markers

A few polypeptides of *Mr* 58 kDa, 45 kDa, 21 kDa and 14 kDa appeared as lightly stained bands. The band of *Mr* 65 kDa disappeared at drought level L1 (10% moisture content) but reappeared at the L2 (5% moisture content) level of drought. As moisture content decreased from L1 to L2, polypeptides of Mr 26 kDa and 14 kDa reduced in intensity (from 1.58 to 1.53 and 1.20 to 1.17), and the rest remained almost unchanged.

In the control treatment, a single intense and darkly stained band of *Mr* 15 kDa represented albumins. As moisture content decreased from 10% to 5%, the intensity of the polypeptide became low (from 1.81 to 1.39) (Fig. 4.1-4.3). In the glutelin fraction, polypeptides were found between *Mr* 12 kDa and 68 kDa at the optimal moisture content (Fig. 6.1-6.3). Polypeptides

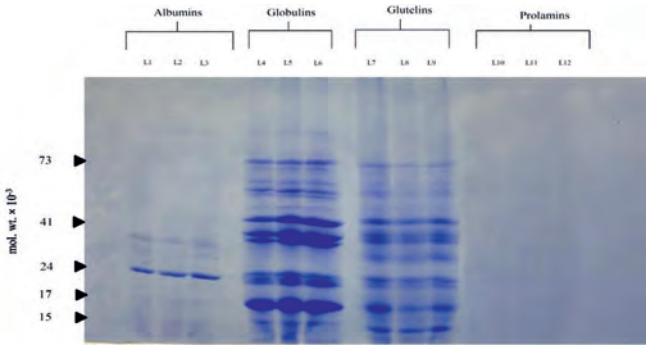

Fig. 3.3. SDS-PAGE of protein fractions from seeds of chickpea genotype HC1 (drought-sensitive) grown under drought stress
L1, L4, L7, L10: Control; L2, L5, L8, L11: 10% moisture; L3, L6, L9, L12: 5% moisture

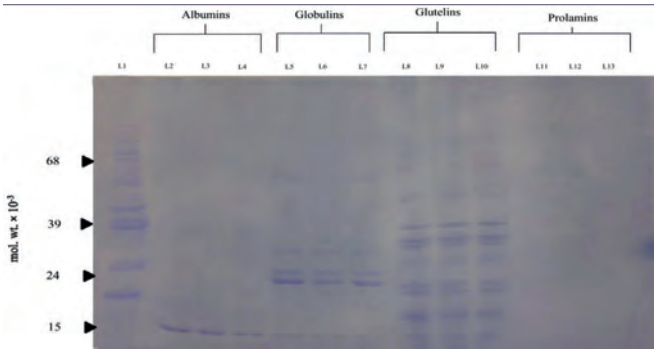

Fig 3.2 SDS-PAGE of protein fractions from seeds of chickpea genotype HC5 (drought-tolerant) grown under drought stress.
L1: Total protein; L2, L5, L8, L11: Control; L3, L6, L9, L12: 10% moisture; L4, L7, L10, L13: 5% moisture

with *Mr* 39 kDa and 34 kDa were intense (1.44 and 1.41) and darkly stained, whereas polypeptides with *Mr* 32 kDa, 22 kDa, 20 kDa and 17 kDa were sharp (0.93, 1.45, 1.57 and 1.30) but lightly stained. The rest of the bands were very stained lightly. As moisture content was reduced from 10% to 5%, the intensity of polypeptide with *Mr* 68 kDa increased (0.97 to 1.21), while *Mr* 28 kDa and 12 kDa polypeptides turned lighter with few variations in intensity (1.40 to 1.38 and 1.39 to 1.13). The polypeptide with *Mr* 51 kDa disappeared when soil moisture content started decreasing. No polypeptide appeared as the new one. The polypeptides in the minor fraction, prolamins did not appear on the gel because of the very low content of this fraction in chickpea seed proteins.

In the sensitive genotype (Fig. 3.3), globulins comprised *Mr* 15 kDa to 100 kDa polypeptides (Fig. 8.1-8.3). Variations in intensities of polypeptides of four fractions of drought-sensitive genotype are compared in Fig. 7.4, 8.4 and 9.4. In the control, the predominant polypeptides of *Mr* 73 kDa, 54 kDa, 41 kDa, 36 kDa, 32 kDa, 24 kDa, 23 kDa and 20 kDa were intense (1.57, 1.48, 2.50, 2.88, 3.16, 2.78, 5.83 and 2.13) and darkly stained. In contrast, those of *Mr* 68 kDa, 60 kDa and 15 kDa were sharp but lightly stained (0.63, 1.27 and

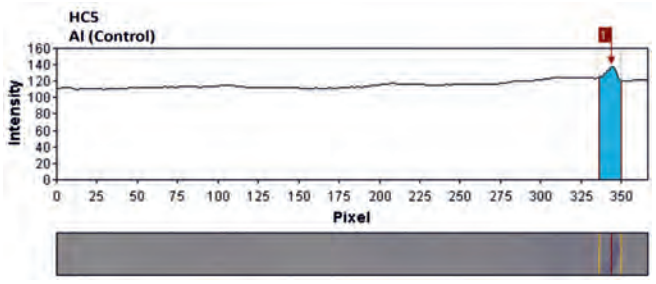

Fig. 4.1. Intensity of albumins (control) in drought tolerant genotype

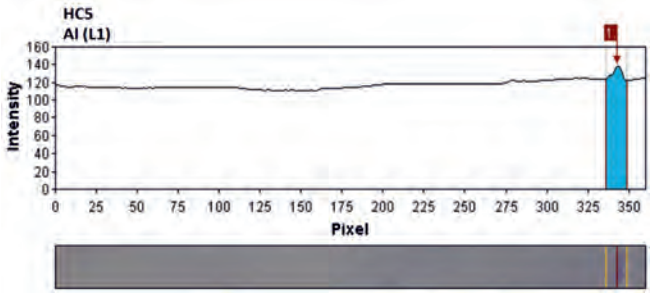

Fig. 4.2. Intensity of albumins (L1) in drought tolerant genotype

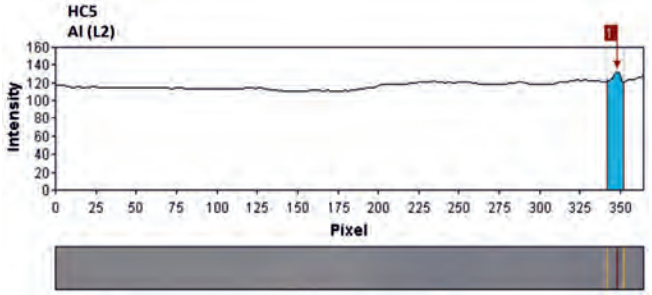

Fig. 4.3. Intensity of albumins (L2) in drought tolerant genotype

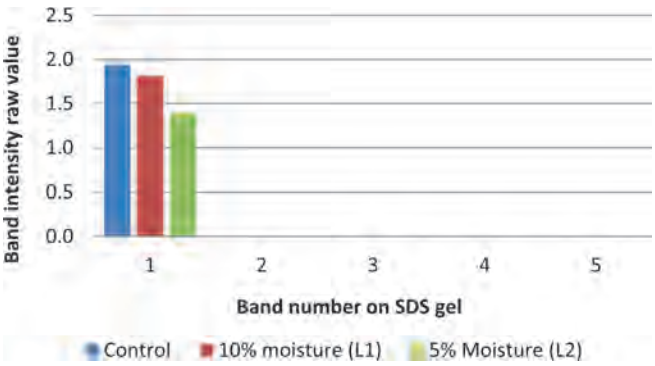

Fig. 4.4. Comparison of bands intensity of albumins in drought tolerant genotype

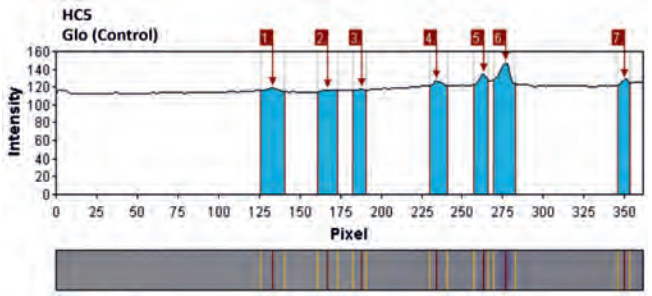

Fig. 5.1. Intensity of globulins (control) in drought tolerant genotype

0.90). The polypeptide of *Mr* 100 kDa was less intense (0.91) and lightly stained. As the level of the drought was raised from L1 to L2 (10% to 5%), polypeptides with *Mr* 100 kDa, 41 kDa, 36 kDa, 24 kDa, 23 kDa, and 20 kDa (0.78 to 1.02, 3.56 to 3.55, 3.68 to 3.48, 3.04 to 2.11, 6.79 to 6.05 and 1.91 to 2.26) became darker. As the level of the drought was changed from L1 to L2 or moisture content was decreased from 10% to 5%, a new globulin band of *Mr* 118 kDa was noticed. Polypeptides of *Mr* 13 kDa to *Mr* 35 kDa were represented by albumins (Fig. 7.1- 7.3). In control, the polypeptide with *Mr* 24 kDa (1.85) was intense and darkly stained. All other bands, *Mr* 35 kDa, 31 kDa, 19 kDa, 15 kDa and 13 kDa, were low-intensity bands (1.52, 1.24, 0.77, 1.15 and 0.63). New albumin polypeptides of *Mr* 102 kDa and 90 kDa appeared when the moisture content was reduced to 5%. The band with *Mr* 35 kDa became lighter (1.87 to 1.44), while *Mr* 24 kDa polypeptide became darker (1.85 to 2.17) as drought stress was increased from L1 to L2. The polypeptides within glutelins exhibited a molecular weight range of 12 kDa to 70 kDa in control (Fig. 9.1- 9.3). Polypeptides with *Mr* 41 kDa, 36 kDa, 22 kDa, 17 kDa and 13 kDa were prominent (2.72, 2.53, 2.35, 4.48 and 2.54). *Mr* 70 kDa and 28 kDa were intense (2.05 and 2.89) but lightly stained. On the other hand, polypeptides with *Mr* 66 kDa, 57 kDa, 53 kDa, 20 kDa, 15 kDa and 12 kDa (0.64, 2.28, 0.88, 1.29, 0.85 and 1.05) were lightly visible on the SDS gel. With the increasing level of drought from L1 to L2, the intensity of polypeptides with *Mr* 70 kDa, 57 kDa, and 53 kDa

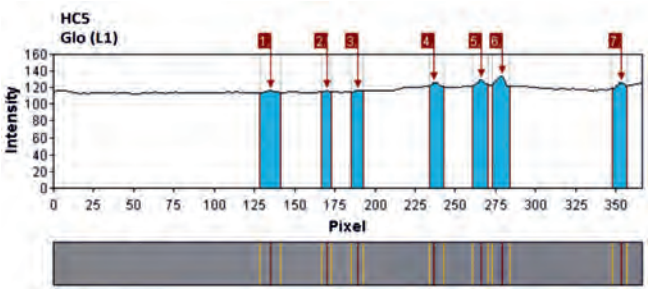

Fig. 5.2. Intensity of globulins (L1) in drought tolerant genotype

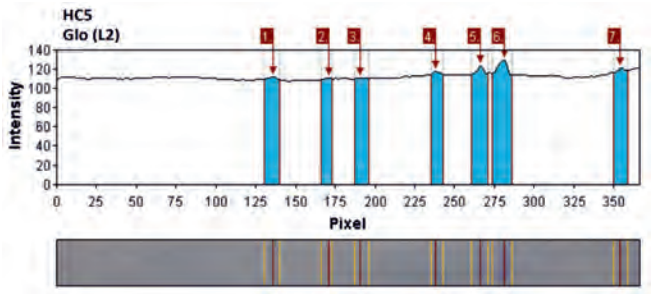

Fig. 5.3. Intensity of globulins (L2) in drought tolerant genotype

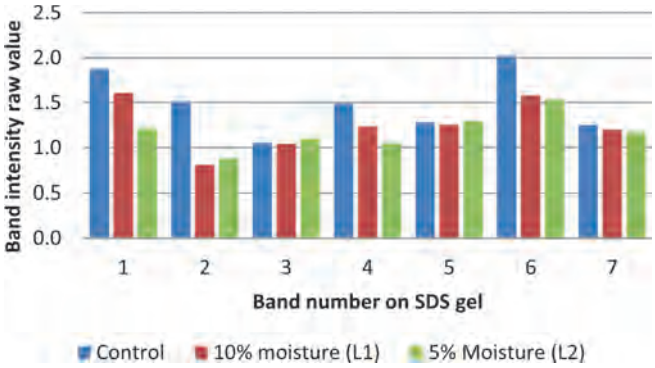

Fig. 5.4. Comparison of bands intensity of globulins in drought tolerant genotype

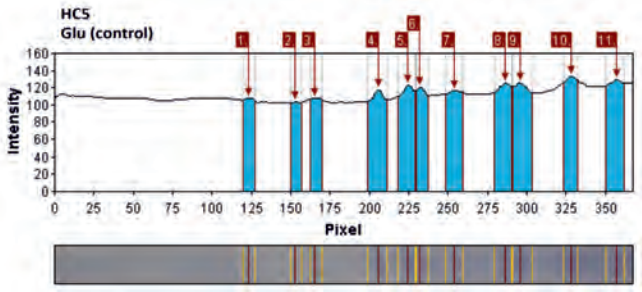

Fig. 6.1. Intensity of glutelins (control) in drought tolerant genotype

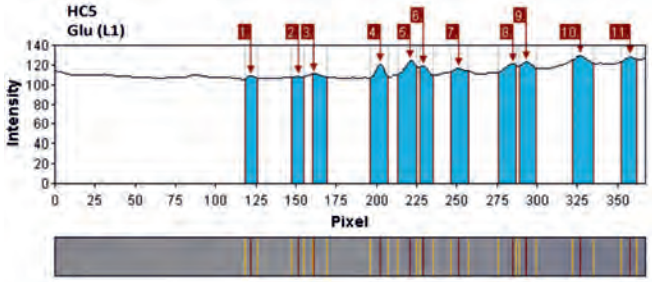

Fig. 6.2. Intensity of glutelins (L1) in drought tolerant genotype

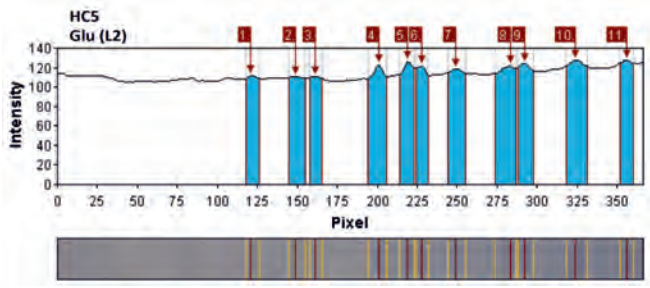

Fig. 6.3. Intensity of glutelins (L2) in drought tolerant genotype

declined (1.42 to 1.31, 2.0 to 1.72 and 0.93 to 0.92), while that of *Mr* 66 kDa increased (0.88 to 1.01). In contrast, bands with *Mr* 36 kDa, 32 kDa, 22 kDa, 20 kDa, and 16 kDa that got reduced (2.68 to 2.75, 1.42 to 1.56, 2.16 to 2.35, 1.37 to 1.45 and 3.47 to 4.30 respectively) at 10% moisture turned darker at 5% moisture level in the soil. Prolamins were not observable on the gel under any treatment.

In consonance with our study, minor changes have also been observed in seed proteins profiles of other crops like groundnut (Javid *et al*., 2004) and chickpeas (Iqbal *et al*., 2005) and leaf proteins of cotton (Parida *et al*., 2007), maize (Jiang and Zhang, 2002), potato (Pruvot *et al*., 1996), vitex (John De Britto, 2011), ashwagandha (Kannan and Kulandaivelu, 2011), etc., in response to drought stress. These little alterations reflect that proteins are affected only slightly under moderate drought stress, as has also been reported by Parida *et al.* (2007). Therefore, no major changes (except intensity) were visible on the gel under increasing drought conditions. The changes in band intensity under drought stress might be due to either up-regulation or down-regulation of genes for these polypeptides (Ingram and Bartels, 1996). The absence or presence of few polypeptides, especially of higher molecular weight, indicates the functional involvement of those polypeptides in response to drought stress

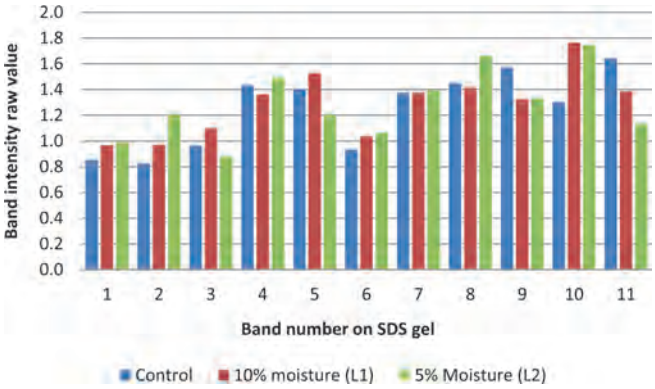

Fig. 6.4. Comparison of bands intensity of glutelins in drought tolerant genotype

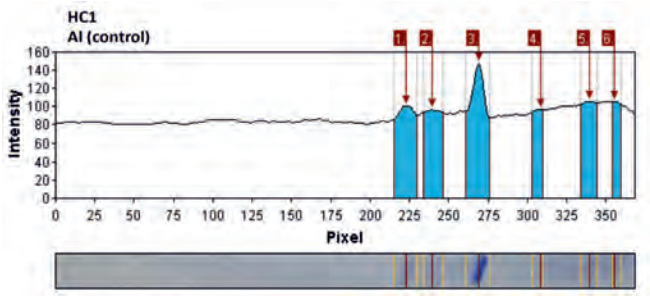

Fig. 7.1. Intensity of albumins (control) in drought sensitive genotype

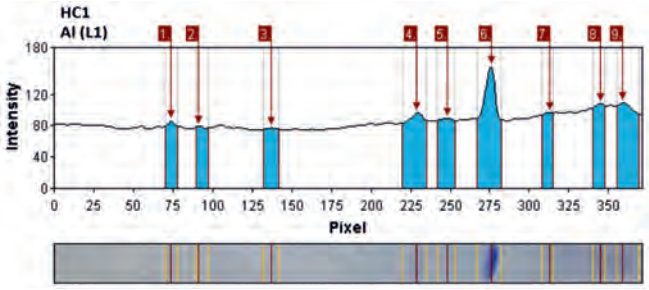

Fig. 7.2. Intensity of albumins (L1) in drought sensitive genotype

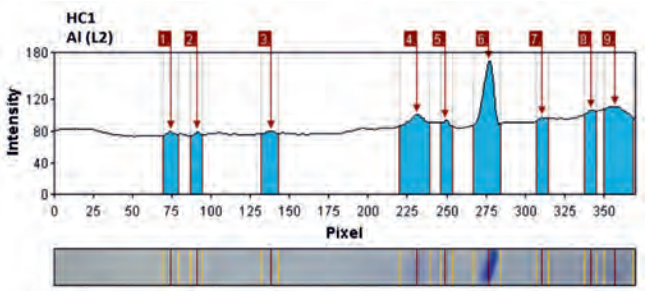

Fig. 7.3. Intensity of albumins (L2) in drought sensitive genotype

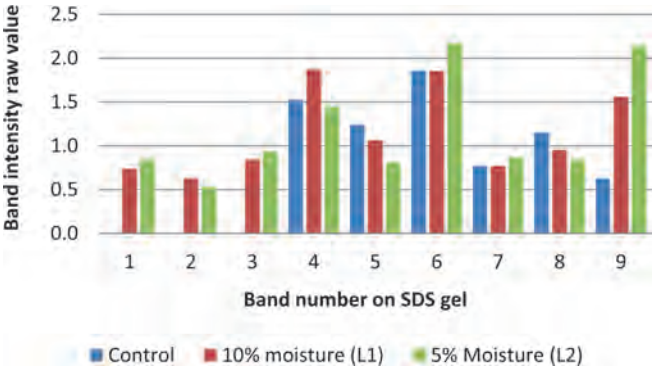

Fig. 7.4. Comparison of bands intensity of albumins in drought sensitive genotype

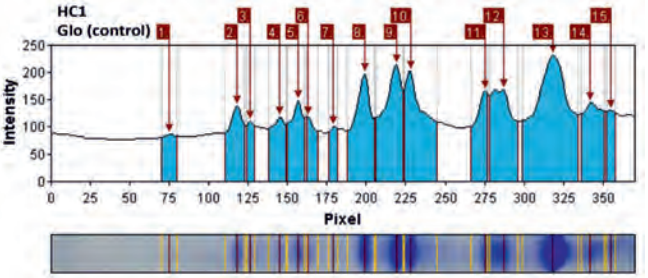

Fig. 8.1. Intensity of globulins (control) in drought sensitive genotype

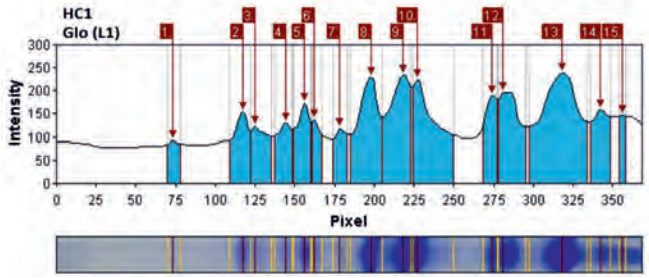

Fig. 8.2. Intensity of globulins (L1) in drought sensitive genotype

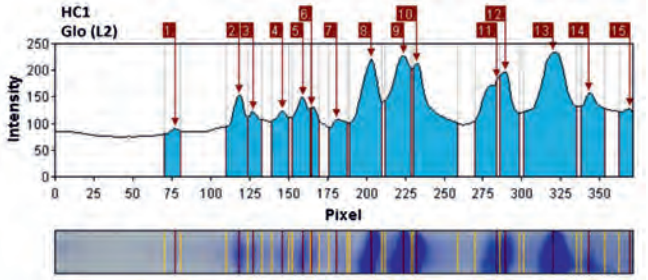

Fig. 8.3. Intensity of globulins (L2) in drought sensitive genotype

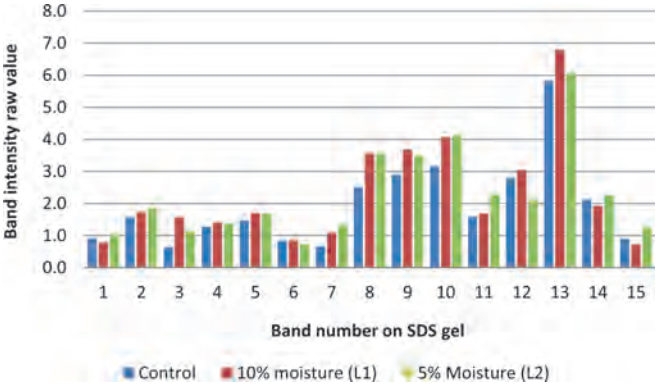

Fig. 8.4. Comparison of bands intensity of globulins in drought sensitive genotype

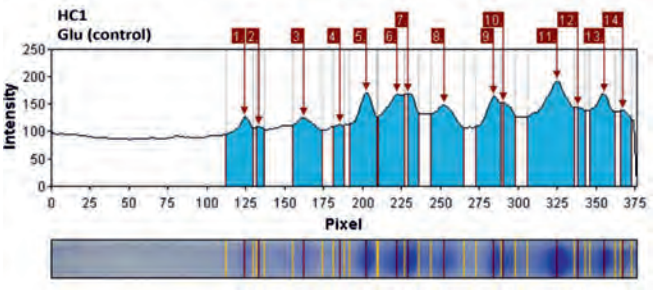

Fig. 9.1. Intensity of glutelins (control) in drought sensitive genotype

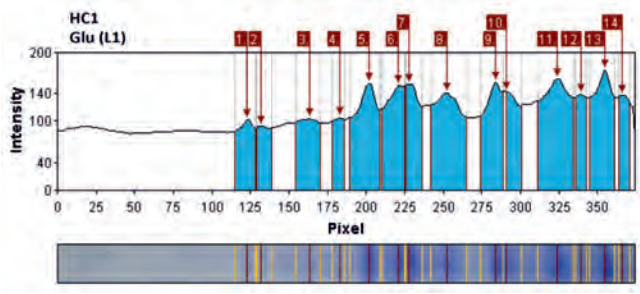

Fig. 9.2. Intensity of glutelins (L1) in drought sensitive genotype

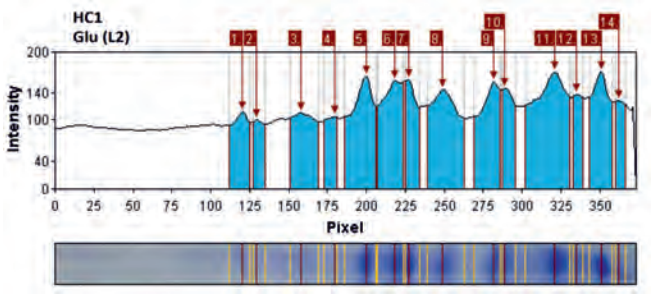

Fig. 9.3. Intensity of glutelins (L2) in drought sensitive genotype

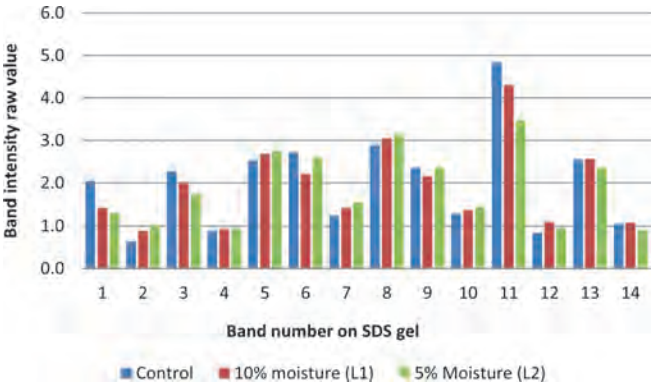

Fig. 9.4. Comparison of bands intensity of glutelins in drought sensitive genotype

(Arefian *et al*., 2014). Also, protein accumulation is less responsive than carbohydrate deposition, resulting in fewer variations in protein assimilation (Elham *et al*., 2012).

Also, the chickpeas subjected to drought exhibited decreased evaporation and stomatal conductance, which may allow the plant to retain water during the reproductive stage (Rani *et al*., 2020). Plants synthesize proteins in response to stress, and most stress proteins are soluble in water; therefore, cellular hydration is crucial to stress tolerance (Viswanathan *et al*., 2004). Free radical scavengers, detoxifying enzymes, water-channel proteins, mRNA binding proteins, anti-freeze proteins, chaperones, osmoprotectants, enzymes for osmolyte synthesis, etc., have been found to increase under drought stress (Bray, 2002). The expression of genes encoding lipid transfer proteins (LTPs) and LEA proteins have also been observed to alter in response to drought stress (Gao *et al*., 2008). Micromics methods can also yield information about the regulation of gene expression at post-transcriptional stage during seed filling by identifying different microRNAs (miRNAs). However, the assessment concerning the impacts of miRNAs during grain filling under drought stress are considerably limited. Further investigations are imperative to elucidate how miRNAs regulate seed filling under drought stress. In arid regions, an improvement in crop yield can be achieved using drought-resistant cultivars with higher water-use efficiency (Ulemale *et al*., 2013). Therefore, the development of drought-resistant genotypes, application of modern breeding techniques, and implementation of water conservation measures, such as drip irrigation and mulching, are all viable avenues for finding a solution to the problems caused by drought (Miyauchi *et al*., 2012).

Authors' contribution

Conceptualisation and designing of the research work (YK, NKM); Execution of field/lab work and data collection (SG, DB); Data analysis and interpretation (SG, A); Manuscript preparation (YK, SG).

Declaration

Authors declare that there is no conflict of interest.

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