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Effect of brassinosteroids on protein profiling of salinity succeptible and resistance cultivars of groundnut under salinity stress

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Abstract
Groundnut (*Arachis hypogaea* L.) belong to Leguminaceae family is a nutritious legume for human diet. It is an important oil seed crop grown in India. Several abiotic stresses including salinity alter the growth and development of this sensitive crop.In the present investigation Leaf protein content reduced in under salinity treatment at the different growth stages of groundnut but when applied with brassinosteroids the protein content increased. Maximum protein content of leaf was reported in all the treatments over control at fruiting stage. Proteins Analysis of salinity resistanc and succeptiable varieties of groundnut was done by Polyacrylamide Gel Electrophoresis (PAGE) method in controle and all treatments seeds after harveting stage. Application of Brassinosteroids induced the synthesis of new resistant protein and increased the intensity of the original protein bands and caused the appearance of additional new bands under the salinity condition.

Keywords: Groundnut, Salinity, Brassinosteroids, Protein, SDS- PAGE

1. Introduction
Groundnut (*Arachis hypogaea* L.) is the most important oil seed as well as an exportable agricultural commodity in India. Groundnut seeds contain 50% oil, 25– 30% protein, 20% carbohydrates and 5% fiber which has high nutritive value for human and produced different types of food like cake as well as the green leafy hay for livestock (Salwa, 2010) [48]. It is widely used as cooking oil, digestible protein, minerals and vitamins in many different countries and contributes significantly to food security and alleviating poverty. However, India, Nigeria, Brazil and Argentina, china, Indonesia, Senegal, USA are major producing countries of groundnut (FAO, 2003) [20]. Among different countries, India has second largest production of groundnut.

Groundnut is one of the most important legumes crops with its largest area in the world, but the area and production of this crop is fluctuating between 6.0 – 8.5 m ha and 6.0 – 9.5 million tones, respectively (Singh and Basu, 2004) [53]. Salinity is one of the most important abiotic stress and limiting factor for worldwide plant production (De bez *et al.*, 2006) [12]. In India soil salinity is around 7.1 m ha, in which 3.5 m ha is under high salinity threat area and 3.5 m ha is under medium level of Salinity (Yadav, 1979) [63]. Major groundnut growing states of India, Soil salinity spread in about 2.0 m ha of coastal and saline areas are affecting the groundnut productivity (Chhabra and Kamra, 2000) [10]. Depends on the variety of groundnut salinity stress affect the seed germination, seedling length; dry matter production and change in ionic concentration etc., (Nithila, 2013) [41]. Salinity stress induced the Ca, K and Fe deficiencies in groundnut (Singh *et al.*, 2004) [53] causing loss of the yield (Hunshal *et al.*, 1991) [26]. Salinity stress also affects the plant metabolic processes such as, photosynthesis, protein synthesis, nitrogen fixation, energy and lipid metabolism (Parida and Das, 2005) [43]. Salinity connects with plant in two ways: Osmotic stress and ion toxicity (Munns, 2002) [39]. Osmotic stress is caused by ions (mainly Na^+ and Cl^-) in the soil solution reducing the water availability to roots. Ion toxicity arise when plant roots take up Na^+ and Cl^- and these ions accumulated to detrimental levels in leaves. Ion imbalances and nutrient deficiency, particularly for K^+ nutrition, can be also occur (Tejera *et al.*, 2007) [56]. In addition, the high level of Na^+ also causes the secondary responses in plants; consequently the oxidative stress is occurred leading to cellular damages in the plant cells (Apel and Hirt, 2004) [4]. This stress can induce over production of reactive oxygen species (Ashraf, 2009) [9]. ROSs derived from the molecular oxygen can accumulate in the plant cell and cause oxidative damages in cellular components, including DNA mutation, proteins, chlorophylls degradation and lipids by lipid peroxidation. Plants commonly react to salinity stress by accumulation of compatible solutes, such as proline, in cells which results in the improvement of environmental stress tolerance in plant (Ashraf and Foolad, 2007) [8].These solutes can be accumulated in high concentrations without impairing plant metabolisms. Over accumulation of these osmolytes may help plants to tolerate against stress by improving their ability to maintain osmotic balance within the cell (Apse and Blumwald, 2002) [5]. Plant cells have the ability to prevent water loss and to maintain the continuous growth in their whole life cycle.

Deleterious effect of salinity stress and increase the resistance to environmental stresses are often correlated with an efficient antioxidative system. Such systems may be induced or enhanced by the application of hormones such as Brassinosteroids. Brassinosteroids are a six group of plant steroidal hormones that regulate various aspects of plant growth and development, including seed germination, cell elongation, photo morphogenesis, xylem differentiation, gene expression regulation, proton pump activation, and induction of ethylene biosynthesis, (Sasse, 2003) [50], as well as adaptation to abiotic and biotic environmental stresses (Divi and Krishna, 2009) [15]. Brassinosteroids are a novel polyhydroxylated steroidal lactone with high growth-promoting activity, obtained from rape pollen (*Brassica napus*

L.) was discovered by Grove in 1979. Brassinosteroid compounds are widely distributed throughout the reproductive and vegetative plant tissues. BRs generally found in reproductive organs such as pollen and fruits in relatively high amounts in seeds (Symons *et al.*, 2008) [55], At present, 70 analogs have been identified, and among these, three brassinosteroids (brassinolide, 24-epibrassinosteroid (EBL), and 28- homo brassinosteroids (HBL) are known to have an economic impact on plant metabolism, growth and productivity, and experience high stability and good performance under field conditions (Khripach *et al.*, 2000) [28]. These compounds are involved in diverse physiological processes such as stem elongation, leaf bending, induction of ethylene, and biosynthesis of nucleic acids and protein, and they also speed the rate of photosynthesis (Khripach *et al.*, 2003; Sasse 2003; Yu *et al.*, 2004) [29, 50, 64]. In addition to these features, BRs (HBL/EBL) are also recognized to have an ameliorative role in plants subjected to various stresses such as those induced by salts, water, drought, low and high temperatures, and heavy metals (Ali *et al.*, 2008; Hasan *et al.*, 2008) [1, 24]. Brassinosteroids are also enhanced the rubisco activity significantly increase the maximum quantum yield of photo system II, and photosynthetic capacity in plants (Yu *et al.*, 2004) [64]. Brassinosteroids also regulate the antioxidant enzyme activity and alleviate the high production of reactive oxygen species and formation of antioxidance defense system in plants against salinity stress condition (Eetezaz Nafie, 2014) [17].

Most of the biochemical studies have been carried out on seed proteins of groundnut by involving their qualitative aspects. Proteomics is becoming a powerful tool to study biochemical pathways and the complex response of plants to different environmental stress. Proteomics also makes an essential connection between the transcriptome and metabolome (Wang *et al.*, 2004; Gray and Heath, 2005) [61, 22], complementing genomics research. Upon several stress responses protein, protein-protein interaction and post translation modification have been also identified by the proteomics (Salekdeh *et al.*, 2002) [47]. Traditionally, Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a qualitative tool used for separation of protein and analysis the molecular weight determination of seed proteins (Luo *et al.*, 2004) [35]. Understanding the seed protein became the focus of studies as the gene expression in plant because the represent the abundant gene products which produced different types of protein during stages of seed development. These seed proteins also offer to basic plant biologists a model for temporal and tissue specific regulation studies during seed development stages (Muller, 1983) [38]. The aim of Present studies is an approach to develop molecular tools protein profiling of groundnut varieties against salinity stress by the application of Brassinosteroids.

2. Material and Methods

Genetically pure seeds of groundnut cultivars were obtained from National Research Centre Center of Groundnut (NRCG), Junagadh, Gujarat. All cultivars were developed with different treatment viz., control, 24-Epibrassinosteroid, 28-Homobrassinosteroids, 24-Epibrassinosteroid+ salinity, 28-Homobrassinosteroids+salinity and conduct the field experiments at the Department of Life sciences, HNGU Patan.

2.1 Estimation of Protein (Lowry *et al.*, 1951) [34]

Collect 0.5 gm leaves sample from fresh seedling at different Growth stages Vegetative, Flowering, Fruting and Harvesting. After collections of leaves crush in 0.1 N NaOH and centrifuge at 10,000 rpm for 20 minutes at 4**.** C. then take the supernant for the protein estimation.

2.2 Analysis of total soluble proteins in seeds through Poly acrylamide gel electrophoresis (PAGE)

Total soluble proteins in the seed were separated using SDS-PAGE technique, following the method of Laemmli (1970) using 12% (w/v) separating gel and 5% (w/v) stacking gel.

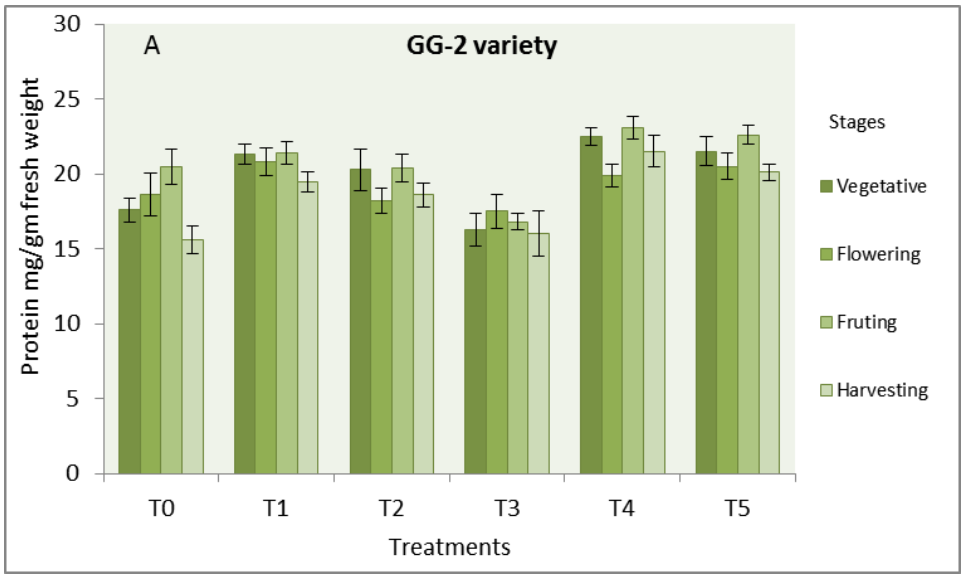
2.3 Extraction of seed proteins

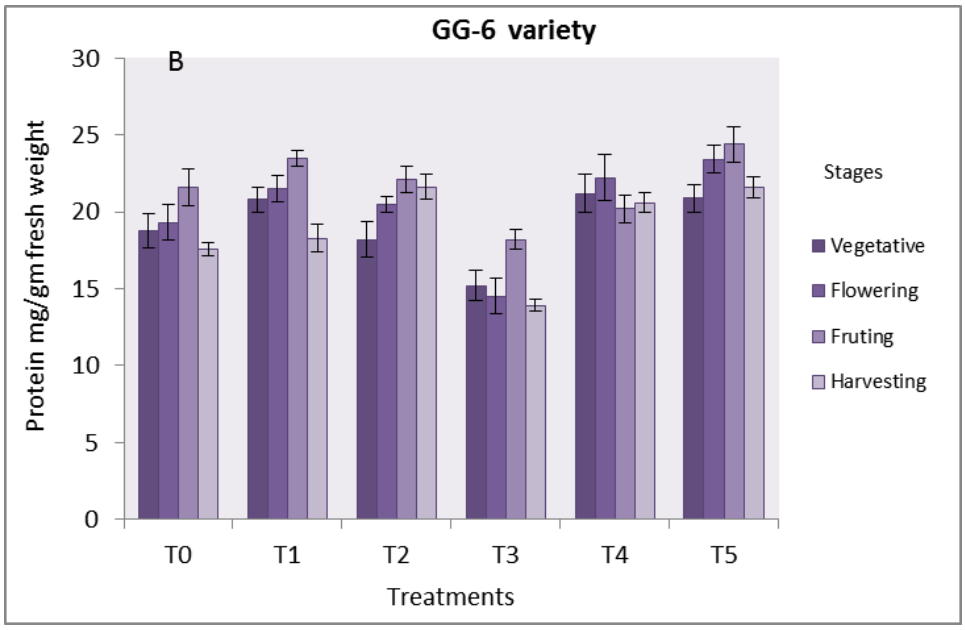
Sodium dodocyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to study the protein profiles of groundnut cultivars like GG -2 and GG-6 (Salinity resistances) and Girnar- 2 and TMV -13 (Salinity susceptible). Seed treated with the extraction buffer which is made up of 2 % PVP, 1mM ascorbic acid, and 5mM EDTA kH_2PO_4 50mM $_PH$ -7. Then centrifuge the sample at 10,000 rpm for 15 minutes and collect the supernant. The supernatant containing the total soluble proteins used to prepare samples for SDS-PAGE and check the concentration of protein in supernant by folin lowery method using bovine serum albumin as standard. (Lowry *et al.*, 1951) [34]. Supernant mix with proper loading dye and then loaded into SDS-PAGE gel. A Pre-stained Protein Ladder (Hi-media 11–245 kDa) was used as molecular weight standard.

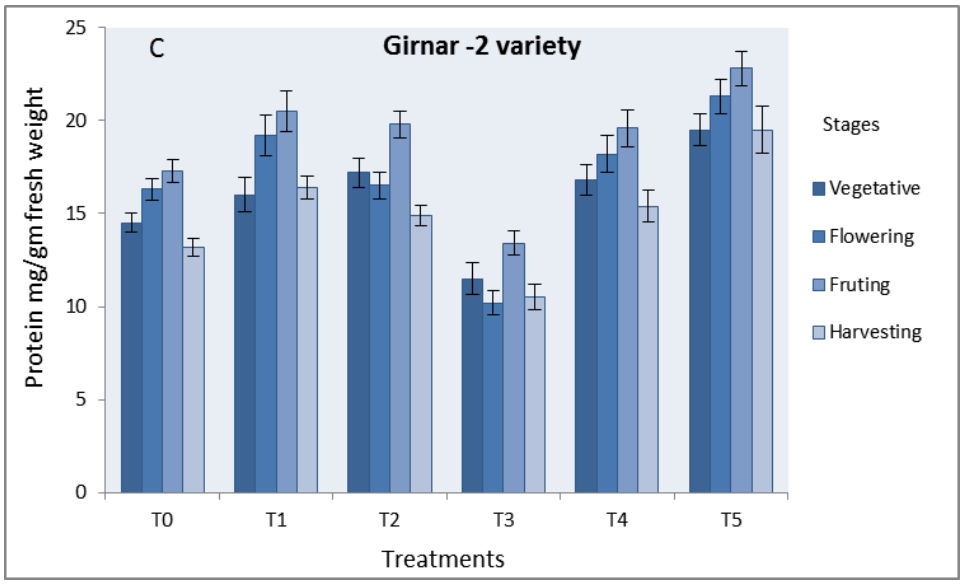
2.4 Electrophoresis

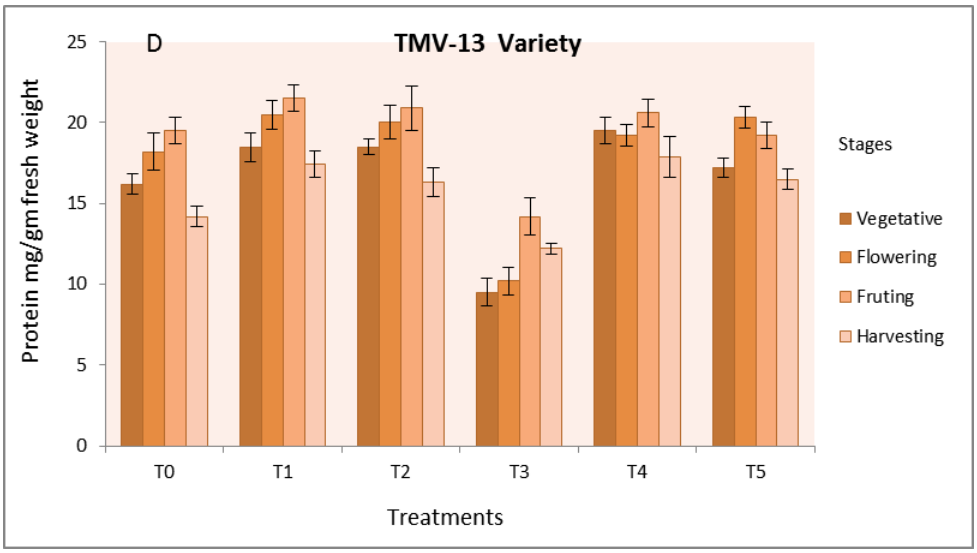
Electrophoresis was carried out at constant voltage of 80 volts until the bromo phenol blue dye reached the resolving gel and then continued at 150 V till the running end and the apparatus was switched off. The gels were dipped in staining solution for one hour at room temperature under gentle shaking condition. Then the staining solution was discarded and the gel was washed once with distilled water and distained for 2 h under shaking condition, replaced every 30 min until bands appeared.

3. Results and discussion









Note : T_o - control,T_1 - 24 - epibrassinosteroid.T_2 – 28 - Homobrassinosteroid, T_3- Salinity, T_4 - 24 – epibrassinosteroid+ salinity, T_5- 28 – Homobrassinosteroid +salinity.

Fig 1: Effect of brassinosteroids on leaf protein content of groundnut cultivars at different stages of growth under salinity stress

Results of Protein content: Figure - 1 show that Significant differences in the leaf protein content were observed in the four groundnut cultivars, during various growth stages.

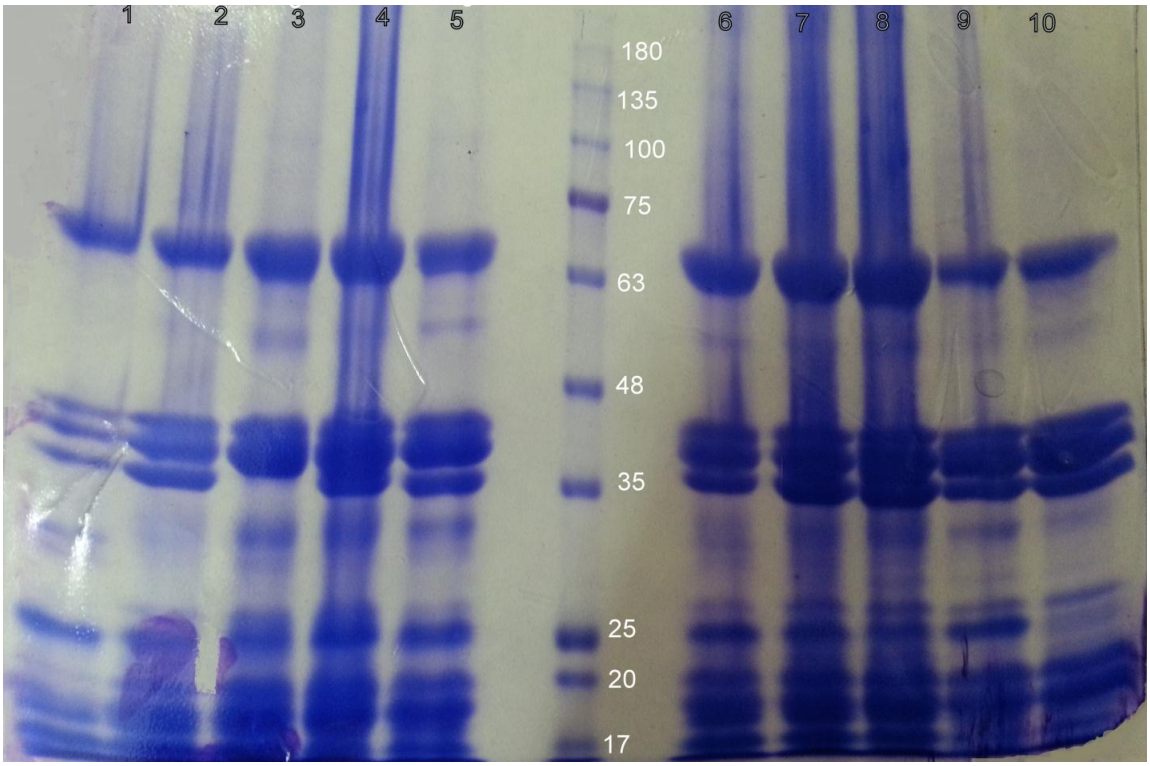
GG-2: In salinity resistances cultivar significant increased in leaf protein content was observed in 24-epibrassinosteroid and 28-Homobrassinosteroid with salinity compared to other treatments. Low leaf protein was reported in salinity compared to control leaf. Among the four stage of development fruiting stage had maximum leaf protein than other stages in control and all the treatments.

GG-6: In salinity resistances cultivar significant increased in leaf protein content was observed in 24-epibrassinosteroid and 28-Homobrassinosteroid with salinity compared to other treatments. Low leaf protein was reported in salinity compared to control leaf. Among the four stage of development fruiting stage had maximum leaf protein than other stages in control and all the treatments.

Girnar–2: In salinity susceptible cultivar significant increased in leaf protein content was observed in 28-Homobrassinosteroid with salinity compared to other treatments. Low leaf protein content was reported in salinity compared to control leaf. Among the four stage of development fruiting stage had maximum leaf protein than other stages in control and all the treatments.

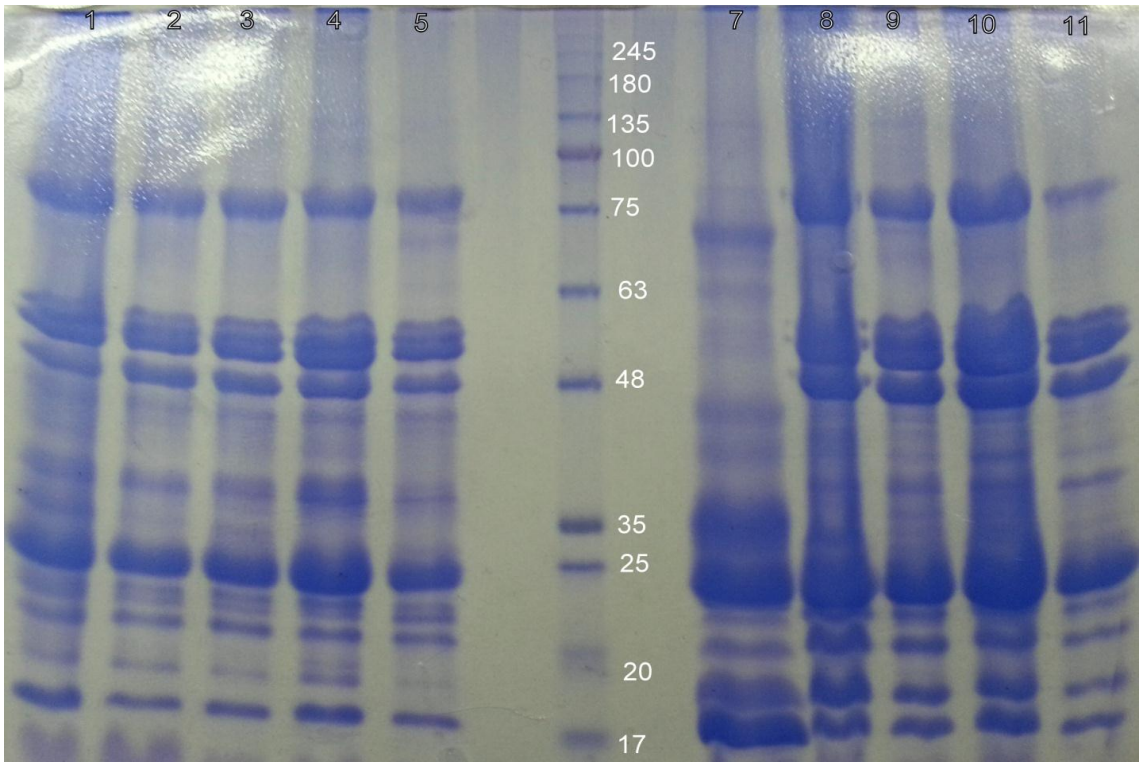
TMV-13: In salinity susceptible cultivar significantly increased in leaf protein content was observed in 24-epibrassinosteroid and 28-Homobrassinosteroid with salinity compared to other treatments.Low leaf protein content was reported in salinity compared to control. Among the four stage of development fruiting stage had maximum leaf protein than other stages in control and all the treatments.

In salinity resistances cultivars salinity slightly affect the protein concentration while the salinity susceptible cultivars greatly affect the protein concentration. Sequential increased in protein content was reported in vegetative, flowering and fruiting stages but it slightly decreased in the harvesting stage in all the treatments. Maximum protein content was reported in Salinity resistances (GG-2, GG-6) compared to Salinity susceptible (Girnar-2, TMV-13) varieties in all the stages of leaf developments. Over all leaf protein content was maximum during fruiting stages in GG-6 cultivar due to 28-homobrassinosteroid with salinity treatments. Salinity stress reduced the protein synthesis and enhance the proteolytic enzyme activity, decreased the amino acid biosynthesis and interferes with tertiary and quaternary structure as a result of that reduced the protein content (Dubey and Rani, 1989) [16]. Wagner *et al.*, (2004) [60] suggested that the amino acid of proteins will react with active radical and it may be degraded. Similar result found that application of Sulfur increased the leaf protein content at different stages on groundnut cultivars (Arshad Jama *et al.*, 2006) [6]. Mehri (2009) [37] Observed that 24- epibrassinosteroid application increased the protein content in normal and stressed *Lycopersicon esculentum* plants. BRs participate in the different processes of gene expression, transcription, and translation in both normal and stressed plants (Sasse, 1999; Mazorra *et al.*, 2002) [49]. Application of BRs increased the protein concentration in tomato leaves (Dhaubhadel *et al.*, 1999; Ogweno *et al.*, 2008) [14, 42] and brome grass (Kulaeva *et al.*, 1991) [30] under high temperature stress which is associated with induction of de novo polypeptide synthesis. Enhanced growth in seedlings by application of BRs under saline conditions was related to enhanced levels of nucleic acids and soluble proteins (Anuradha and Rao, 2001, 2003; Shahbaz *et al.*, 2008) [2, 3, 52]. Sairam (1994) [16] reported that increased the proteins content in wheat plants by homobrassinosteroid under moisture stress conditions. He also noticed that osmotic stress resulted in considerable reductions in the protein content in the seedlings of both susceptible and tolerant varieties of sorghum plants but treatment with brassinosteroids resulted in not only restoring the protein levels but also further improvement (Vardhini & Rao, 2003) [57].



Note: Here, Lane 1 – 5 GG2, Lane 7-11 GG 6 Varieties of groundnut and Lane -6 Protein marker Lane: 1 Control, Lane: 2 28-Homobrassinosteroid, Lane: 3 28-Homobrassinosteroid+Salinity, Lane: 4 24- Epibrassinosteroid Lane: 5 24- Epibrassinosteroid +salinity Lane: 7 Control, Lane: 8 28-Homobrassinosteroid, Lane: 9 28-Homobrassinosteroid +Salinity, Lane: 10 24- Epibrassinosteroid, Lane: 11 24- Epibrassinosteroid+salinity.

Fig 2: Effect of brassinosteroids on protein profiling of GG- 2 and GG- 6 (Salinity resistances) groundnut cultivars under salinity stress.



Note: Here, Lane 1 – 5 Girnar -2. Lane 7-11 TMV - 13Varieties of groundnut and Lane -6 Protein marker Lane: 1 Control, Lane: 2 28-Homobrassinosteroid, Lane: 3 28- Homobrassinosteroid+Salinity, Lane: 4 24-Epibrassinosteroid, Lane: 5 24- Epi brassinosteroid +salinity Lane: 7 Control, Lane: 8 28-Homobrassinosteroid, Lane: 9 28-Homobrassinosteroid +Salinity, Lane: 10 24- Epibrassinosteroid, Lane: 11 24- Epibrassinosteroid+salinity

Fig 3: Effect of brassinosteroids on protein profiling of Girnar - 2 and TMV-13 (Salinity succeptible) groundnut cultivars under salinity stress.

Table 1: Effect of Brassinosteroids on SDS-PAGE protein banding pattern in groundnut varieties GG- 2 and GG- 6 (Salinity resistances) under salinity stress.

Groundnut Varieties		GG -2						GG - 6					
Sr. No	Marker Protein	M.W (KD)	T_0	T_1	T_2	T_3	T_4	M.W (KD)	T_0	T_1	T_2	T_3	T_4
1	17	17	+	+	+	-	+	17	+	+	+	+	+
2	20	19	+	+	-	-	-	18	+	+	+	+	+
3	25	20	+	+	+	+	+	19	+	+	+	+	+
4	35	23	-	-	+	-	-	20	+	+	+	+	+
5	48	25	+	+	+		+	25	+	+	+	+	-
6	63	26	-	-	-	+	-	26	+	+	+	+	+
7	75	32	-	-	+	+	+	28	-	+	+	+	-
8	100	35	+	+	-	+	+	33	-	-	-	+	-
9	135	36	+	+	-	-	-	35	+	+	+	+	+
10	180	37	+	+	+	-	+	37	+	+	+	+	-
11		38	+	+	-	-	-	39	+	+	-	+	+
12		52	-	-	+	-	+	65	+	+	+	+	+
13		65	+	+	+	+	+						
Total Bands		13	9	9	8	5	8	12	10	11	10	12	8

Note: M.W= molecular weight, T_0– Control, T_1- 28-Homobrassinosteroid, T_2- 28-Homobrassinosteroid +Salinity, T_3- 24- Epi brassinosteroid. T_4- 24- Epibrassinosteroid +salinity, M.W = molecular weight, T_0 - Control. T_1 - 28-Homobrassinosteroid, - T_2 - 28 - Homobrassinosteroid+Salinity. T_3 - 24-Epibrassinosteroid, T_4-24- Epibrassinosteroid +salinity

Table 2: Effect of Brassinosteroids on SDS-PAGE protein banding pattern in groundnut varieties Girnar -2 and TMV -13 (Salinity succeptible) under salinity stress.

Groundnut Varieties		Girnar -2						TMV -13					
Sr. No	Marker Protein	M.W (KD)	T_0	T_1	T_2	T_3	T_4	M.W (KD)	T_0	T_1	T_2	T_3	T_4
1	17	18	+	+	+	+	+	18	+	+	+	+	+
2	20	19	+	+	+	+	+	19	+	+	+	+	+
3	25	21	+	+	+	+	+	21	+	+	+	+	+
4	35	24	+	+	+	+	+	24			+	+	+
5	48	25	+	+	+	+	+	25	+		+	+	
6	63	37	+	+	+	+	+	37	+				
7	75	45	+	+	+	+	+	40		+	+	+	+
8	100	48	+	+	+	+	+	42				+	
9	135	58	+	+	+	+	+	48	+	+	+	+	+
10	180	73					+	60			+	+	+
11	245	75	+	+	+	+	+	63	+				
12								73	+				
13								75		+	+	+	+
Total Bands		11	10	10	10	10	11	13	8	6	9	10	8

Note: M.W= molecular weight, T_0– Control, T_1- 28-Homobrassinosteroid, T_2- 28-Homobrassinosteroid +Salinity, T_3- 24- Epi brassinosteroid. T_4- 24- Epibrassinosteroid +salinity, M.W = molecular weight, T_0 - Control. T_1 - 28-Homobrassinosteroid, - T_2 - 28- Homo brassinosteroid +Salinity. T_3 - 24-Epibrassinosteroid, T_4-24- Epibrassinosteroid +salinity

Results of protein profiling

Figure: 2 show that the effect of brassinosteroids on protein profiling of salinity resistances groundnut seeds cultivars under salinity stress

GG-2: In GG-2 cultivar SDS-PAGE Electrophoretic protein analysis in term of bands showed that the polypeptides with molecular weights 17, 19, 20,25,35,36,37,38,65 KD were prominent in control and in treatment 28-Homobrassinosteroid (Fig.2 and Table 1). No novel band was seen in 28-Homobrassinosteroid individual treatment but the band intensity showed slight increase in compared to control. 28-Homobrassinosteroid applied with salinity shows 17, 20, 23, 25, 32, 37, 52, 65 KD molecular weight polypeptides and in this treatment two novel band expressed are 32 and 52 KD molecular weight protein. On application of 24-epibrassinosteroids, polypeptides with molecular weights 20, 26,32,35,65 were reported with very high Intensity of protein Bands compared to control. When 24-epibrassinosteroid applied with salinity 17, 20, 25, 32, 37, 52, 65 KD molecular weight polypeptides were reported and 52 KD molecular weight band was seen only in 24-epibrassinosteroid with the salinity. 28-homobrassinosteroid and 24-epibrassinosteroid increased the band intensity and express the novel band in presences of hormones + salinity in GG-2 cultivar but not separately.

GG-6: In GG-6 cultivar SDS-PAGE Electrophoretic analysis in term of bands showed that the polypeptides with molecular weights 17, 18, 19, 20, 25, 26, 35, 39, 65 KD were prominent in control (Fig.2 and Table 1).28-Homobrassinosteroid applied individual and with salinity showed that the polypeptides with molecular weights 17, 18, 19, 20, 25, 26, 28, 35, 37, 39, 65KD were reported. 24-epibrassinosteroids applied individual show

that polypeptides with molecular weights 17, 18, 19, 20, 25, 26, 28, 33, 35, 37, 39, 65 KD were reported. 33 KD molecular weight band present in 24-epibrassinosteroid treatment.When 24-epibrassinosteroid applied with salinity 17, 18, 19, 20, 25, 26, 28, 35, 37, 39, 65 KD molecular weight present.

Figure: 3 show that the effect of brassinosteroids on protein profiling of salinity succeptible groundnut seeds cultivars under salinity stress

Girnar -2: In Girnar-2 cultivar SDS-PAGE Electrophoretic analysis in term of bands showed that the polypeptides with molecular weights18, 19, 21, 24, 25, 37, 45, 48, 58, 75KD were prominent in control, 28-Homobrassinosteroid,28-Homobrassinosteroid with salinity and 24-epibrassino steroids(Fig.3 and Table 2). When 24-epibrassinosteroid applied with salinity 18, 19, 21, 24, 25, 37, 45, 48, 58, 73,75 KD molecular weight polypeptide were reported.73 KD molecular weight polypeptide found only in 24-epibrassinosteroid with salinity.

TMV-13: In TMV-13 cultivar SDS-PAGE Electrophoretic analysis in term of bands showed that the polypeptides with molecular weights 18, 19, 21, 25, 37, 48, 63, 73 KD were prominent in control (Fig.3 and Table 2). 28-Homobrassinosteroid applied individual showed that the polypeptides with molecular weight 18, 19, 21, 40, 48, 75 KD were reported. When 28-Homobrassinosteroidand 24-epibrassinosteroid applied with salinity 18, 19, 21, 24, 25, 40, 48, 60, 75 KD molecular weight polypeptide were reported.24-epibrassinosteroid applied individual show that polypeptides with molecular weight 18, 19, 21, 24, 25, 40, 42, 48, 60, 75 KD were reported. 42 KD molecular weight polypeptide seen only in 24-epibrassinosteroid.

Here, result clearly indicate that brassoinosterods induced and synthesis and increased of original protein bands and caused the appearance of additional new bands under saline and non-saline condition.24-Epibrassinosteroid is more potential than 28- Homobrassinosteroid. When applied separately but with salinity 28- Homobrassinosteroid was found effective seed protein. Similar result found that new protein band formation in *Cyamopsis tetragonoloba* with high molecular weight found in salt stressed plant with sitosterol treatments may be due to *de novo* synthesis of these proteins (Gopala Roa *et al.,* 1987) [21]. These new polypeptide band may have specific function to protect the plant from dehydration damage and resistance against salinity stress (Jiang and Huang, 2002) [27]. Variation in bands intensity of protein may be due to variation in protein solubility or reduced the separation of several proteins having similar migration rates observed in different plant like Chun *et al*., (1994) [11], Asghar *et al.*, (2003) [7] and Varma *et al*., (2005) [58] in maize genotypes, Devi (2000) [13] in sunflower, Vijayan (2005) [59] in rice, Paul and Datta (2006) [44] in celery and ajowan, Nisha (2007) [40] in wheat and Sumathi (2007) [54] in oats. Sevgi, *et al,* (2014) [51] reported that Homobrassinoloid reduced the protein content compared to control but when applied in saline condition increased the protein bands intensity in Barly roots. 24-Epi treatment change in protein synthesis under saline condition may be due to some modifications of expression gene at the mRNA translation or *via* regulation of RNA transcription. Lapeyre *et al*.,(1987) observed that 77.6 KD protein band present may be brassinosteroid synthesis de novo synthesis in *Ph. vulgaris.*(Hassanein, 1999) [32] also observed that the protein profiling of groundnut show127 and 52 KD induced and 260 and 38 KD repress in synthesis of new protein under saline condition. Gomathi *et al.,* 2013 [45] cited that salt resistance protein role as the molecular marker of plant it accumulate and enhanced the salt tolerances and susceptible protein in Sugarcane crop. New protein polypeptide protein 15, 26 and 72 KD present in tolerances and susceptible cultivars due to application of GA3 under saline condition. Appear the new protein bands in presences of GA3 under saline condition may be due to survival and growth of plant. El-Shintinawy and El-Shourbagy (2001) [19] conformed that effect of salinity reduced by application of thymine.2µM thiamine increased and appeared the 24 KD protein in wheat plant which is absent in saline condition. El-Farash *et al.,* (1993) [18] also found that under saline condition 12 different types of polypeptides protein bands appear and expression of these proteins is genetically regulated in tomato plants.

4. Conclusion

The results shows that leaf protein content was found more in salinity + Brassinosteroids treatments during both growth and reproductive stage in all the varieties. Seed protein profiling of resistance varieties GG-2 shows maximum number of bands in 28-Homobrassinosteroid and novel band in 28-Homobrassinosteroid +salinity treatments where as in GG-6 maximum and novel band both in 24-Epibrassinosteroid treatment and polypeptide (65KD) larger size was reported in both varieties. In salinity susceptible varieties Girnar-2 maximum band was reported in 24-Epibrassinosteroid + salinity and TMV-13 maximum number of band was in 24-Epibrassinosteroid treatments and polypeptides (75KD) large size was reported in both varieties. Maximum number of bands and novel band was in 24-Epibrassinosteroid treatments. Overall results show that 24-Epibrassinosteroid is more potential then 28- Homobrassinosteroid. When applied separately but with salinity 28- Homobrassinosteroid was found effective seed protein. Both the 24-Epibrassinosteroid and 28- Homobrassinosteroid were found effective for salinity alleviation but more in salinity resistance varieties compared to susceptible.

5. Acknowledgement

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