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EFFECT OF SEED PRIMING WITH AQUEOUS EXTRACTS OF CARROT ROOTS, GARLIC CLOVES OR ASCORBIC ACID ON THE YIELD OF *VICIA FABA* GROWN UNDER DROUGHT STRESS

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Abstract

Two drought stress treatments were applied on *Vicia faba* (cv. Giza 716) seedlings (14-days-old). Watering was not done for 14 days (D1) or 22 days (D2) and left to grow on clay-sandy soil (2:1 w/w) till yield stage. The impact of seed priming by presoaking in the extract of carrot root (Cr), garlic cloves (G), or ascorbic acid (AA) on the alleviation of the hurtful influence of drought stress was studied. Results showed that drought stress (D1 and D2) caused reduction in glucose, sucrose and starch contents of the increased seeds and in the yield also, while the total soluble protein content was increased. Seed priming with G, Cr or AA reduced the deleterious effects of drought on yield attributes and metabolites of *Vicia faba*, with some enhancements over their corresponding control values. SDS-PAGE of the yielded seeds showed that D1 and D2 led to the appearance of some newly synthesized protein bands and disappearance of others. Priming with G, Cr or AA resulted in the re-appearance of the disappearing bands and the synthesis of new ones. ISSR-analysis of the D2-stressed-yielded seeds showed that priming with G or Cr extracts resulted in the formation of one and two unique bands, respectively. Priming with AA caused the disappearance of one unique band (466 bp) which was restored under priming with G, Cr or AA. Seven newly synthesized bands appeared on priming.

Key words: Ascorbate, Carrot, DNA, Drought, Field bean, Garlic, Proteins, Sugars, Yield.

Introduction

Faba bean (*Vicia faba* L.) is an important legume crop in Egypt, and it categorizes as the fourth most important legume crop in the world, after dry beans, dry peas and chickpeas (Abid *et al.*, 2015). The production of faba bean is not adequate to meet domestic demand, and Egypt is presently the world's largest faba bean importer (Youseif *et al*., 2017). Increasing faba bean production is one of the foremost vital objectives of agricultural policy in Egypt (Dawood *et al.*, 2014).

Drought stress is a major threat to crop production worldwide because it has a negative effect on plant growth and yield (Siddiqui *et al.*, 2015). However, faba bean is sensitive to water stress throughout its developmental stages, particularly at the reproductive stage, where the adverse influences of drought on plant morphology and physiology are coupled with intrinsic depression in the rate of growth and biomass accumulation in the crop (Khan *et al*., 2010; Farooq *et al.*, 2012; Alzahrania *et al*., 2018). Plants display a variety of mechanisms to resist drought such as reduced water loss by raised water uptake with prolific and deep root systems, smaller and succulent leaves to diminish water loss through transpiration, and/or accumulation of cuticular waxes (Farooq *et al*., 2012).

The pernicious actions of various abiotic stresses are often eased by application of natural and artificial growth regulators (Lipiec *et al.*, 2013). This application is often administrated by different ways like spraying or priming of seeds by presoaking in these regulators. Seed priming is a cheap, quick and straightforward methodology accessible to farmers for achieving higher germination rates and may be taken to counteract the adverse effects of abiotic stresses (Reiahi & Farahbakhsh, 2013; Ahmed *et al.*, 2014).

The extract of carrot roots (*Daucus carota* L.) is used in the stimulation of plant growth because it is known to contain a variety of plant growth-stimulating compounds. The analysis of carrot roots by HPLC showed that its extract includes high content of vitamin A as β-carotene, protein, carbohydrates, fat and vitamins B1, B2, B6, C, D and E with notable antioxidant that reduce free radicals and cell injury (Kasim *et al.*, 2017). Similarly, garlic (*Allium sativum* L.) is a crop of worldwide economic importance with a characteristic resistance to biotic and abiotic stresses, such as viral, bacterial, heavy metals, and oxidative stress (Liu *et al.*, 2009). Also, ascorbic acid (vitamin C) provides high protection against drought stress through enhancement of plant growth, cell elongation and cell division (Abbas & Akladious, 2013), regulation of photosynthesis (Yazdanpanah *et al.*, 2011), protecting proteins, lipids and cells against oxidative stress (Hasanuzzaman *et al.*, 2013) and increasing carbohydrates and total nitrogen percent in plants (Athar *et al*., 2008).

The purpose of this research is to assess the influence of priming the seeds of *Vicia faba* with the extracts of carrot roots, garlic cloves (as two natural materials) or with ascorbic acid solution on the yield, under two treatments of drought stress.

Material and Methods

Plant materials: Seeds of *Vicia faba* (cv. Giza 716) were supplied by the Agricultural Research Center, Giza, Egypt, and handpicked for apparent uniformity of size and form. Fresh plants of carrot and garlic were obtained from the local market.

Preparation of the extracts: The extract of carrot (*Daucus carota* L.) roots was prepared according to

Sofowora (1982) with some modifications (Kasim *et al.*, 2017) as 200 g fresh carrot roots/L and considered as 100% extract. The extract of garlic (*Allium sativum* L.) was prepared according to Hanafy *et al.*, (2012) with some slight amendments (Kasim *et al.*, 2017) as 250 g fresh garlic cloves /L and considered as 100% extract. Ascorbic acid solution was prepared with a concentration of 0.5 mM.

Seed cultivation and treatments of drought stress: Depending on the results of a preliminary experiment reported in Kasim *et al.*, (2017), *Vicia faba* seeds were divided into four groups and primed by soaking for 10 hrs either in tap water, 100 % garlic extract (G), 100 % carrot extract (Cr) or 0.5 mM ascorbic acid (AA). For every treatment, 15-primed *Vicia faba* seeds were planted in a plastic pot (40 cm diameter and 45 cm depth) filled with 20 kg of clay-sandy soil (2:1 w/w). Seeds were irrigated with tap water once daily for three days, then twice weekly and left to grow for 14 days under natural environmental conditions (16/8 hrs day/night at 25°C /15°C ± 2 day/night and relative humidity of 65 %). Each group of the 14-days old seedlings was then divided into 3 sub-groups. The first was irrigated with tap water (60% field capacity) twice weekly until the end of the experiment and considered as the control. The second was subjected to water withholding for fourteen days and thought of as initial drought stress D1 treatment. The third sub-group was subjected to water withholding for 22 days and considered as the second drought stress D2 treatment. At the end of each water withholding period, each sub-group was re-irrigated with tap H_2O doubly weekly till the yield stage (160 days).

At the end of the season, the yielded seeds were collected to assess the yield parameters such as number of pods per plant, number of seeds per pod, percentage of mature seeds per pod, mass of pods per plant, mass of seeds per pod, mass of seeds per plant and mass of a thousand seeds. Carbohydrate and total soluble protein contents, protein patterns as well as the ISSR analysis of DNA (for samples of D2 only) were determined in the yielded seeds.

Analysis of the yielded seeds: Soluble sugars and starch contents within the yielded seeds were calculated quantitatively using the Nelson's method (1944) as modified by Naguib (1963 and 1964) and calculated as mg g^{-1} dry wt. Total soluble proteins were extracted following Naguib *et al.*, (1968) and it was determined as described by Bradford (1976) and calculated as mg g^{-1}dry wt.

The protein patterns of yielded seeds were analyzed using continuous Poly Acrylamide Gel Electrophoresis (SDS-PAGE) following Laemmeli (1970). The DNA patterns were studied using PCR analysis and the used conditions were as described in the manual of the Kits by ZR Plant/Seed DNA MiniPrep™; Catalog No. D6020 of ZYMO RESEARCH CORP. (www.zymoresearch.com). The PCR (DNA ladder) markers (M) were 100bp /200 /300 /400/ 500/ 1000 /1500 (promega 100bp DNA Ladder G210A1). The sequences of the five primers assayed in the PCR were as the following:

Primer-No.	Name	Sequence
1	UB-C818	CACACACACACA CACAG
2	UBC-822	TCTCTCTCTCTCTCTCA
3	UBC-823	TCTCTCTCTCTCTCTCC
4	UBC-824	TCTCTCTCTCTCTCTCG
5	UBC-843	CTCTCTCTCTCTCTCTCA

The results were statistically analyzed using one way Analysis of Variance (ANOVA) to elaborate the degree of significance for the obtained variations by the used treatments. The analysis was carried out by COSTAT (6.311) statistical program.

Results

Yield parameters: The D1 treatment resulted 23.7 % and 28.9% decline in the percentages of mature seeds/pod, mass of pods per plant relative to the control, respectively (Fig. 1). Reduction of the same two parameters owing to the D2 treatment was even greater (33 % and 39 %, respectively relative to the control).

Similarly, mass of seeds /pod, mass of seeds/plant and mass of 1000 seeds showed the same trend. Meanwhile, compared to the control, drought caused non-significant change in both pods number per plant and seed number per pod.

The combined treatments of drought stress and priming of *Vicia faba* seeds with G, Cr or AA overcame these inhibitory effects, so that all the measured yield parameters were raised, compared to the drought-stressed plants.

Total soluble protein and Carbohydrates: The two drought treatments D1 and D2 generated a significant boost in total soluble protein with percentages 4.8 % and 7 %, respectively relative to the control. Priming of seeds with G, Cr or AA reversed these increases: with D1 their values became lower than those of the control, but for samples of D2, the values remained higher than those of the control (Fig. 2A).

Figs. 2B, C and D show that drought stress caused reductions in each of glucose, sucrose and starch with percentages of 12.5 %, 9.8 % and 7.8%, respectively with D1, while, with D2 they were 15.6 %, 21.8 % and 15.5 %, respectively relative to the control. Priming of seeds in G, Cr or AA led to a significant amelioration of these harmful effects of drought stress, where the sucrose and starch were increased, while glucose was decreased, compared with the drought stressed samples.

Protein patterns of the yielded seeds: The molecular masses (MM) of protein patterns shown in Table (1) provide the expression of the effects of drought stress and seed priming in the yielded seeds of *Vicia faba*. Data revealed that D1 and D2 induced the disappearance of seven and eight bands, respectively. Each of D1 and D2 induced eight newly synthesized bands that were not detected in the control. Priming of *Vicia faba* seeds with G, Cr or AA not only relieved the deleterious impact of drought stress through the re-appearance of six bands which disappeared under drought stress, but also by the induction of newly synthesized bands (Table 1).

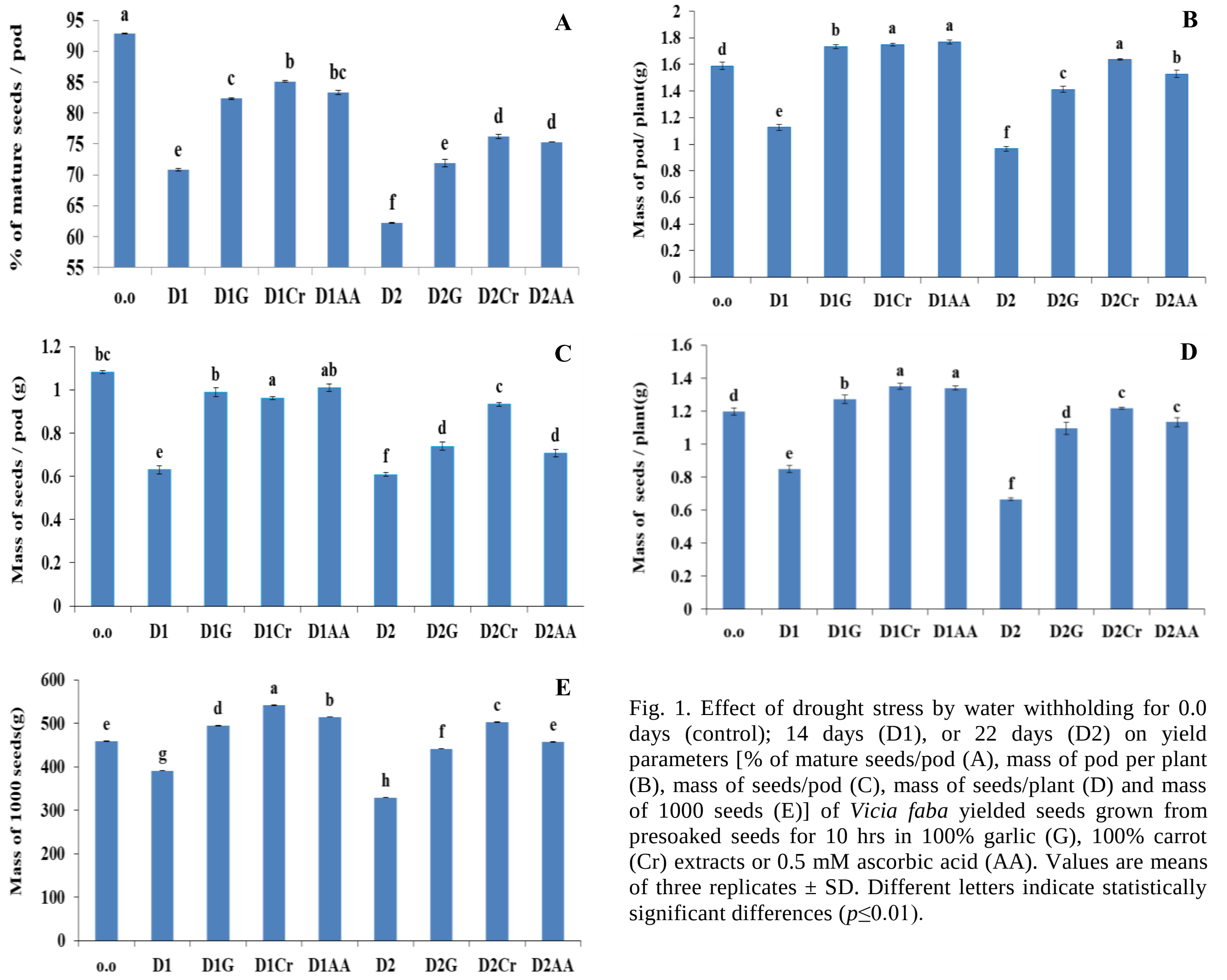

Fig. 1. Effect of drought stress by water withholding for 0.0 days (control); 14 days (D1), or 22 days (D2) on yield parameters [% of mature seeds/pod (A), mass of pod per plant (B), mass of seeds/pod (C), mass of seeds/plant (D) and mass of 1000 seeds (E)] of *Vicia faba* yielded seeds grown from presoaked seeds for 10 hrs in 100% garlic (G), 100% carrot (Cr) extracts or 0.5 mM ascorbic acid (AA). Values are means of three replicates ± SD. Different letters indicate statistically significant differences ($p \leq 0.01$).

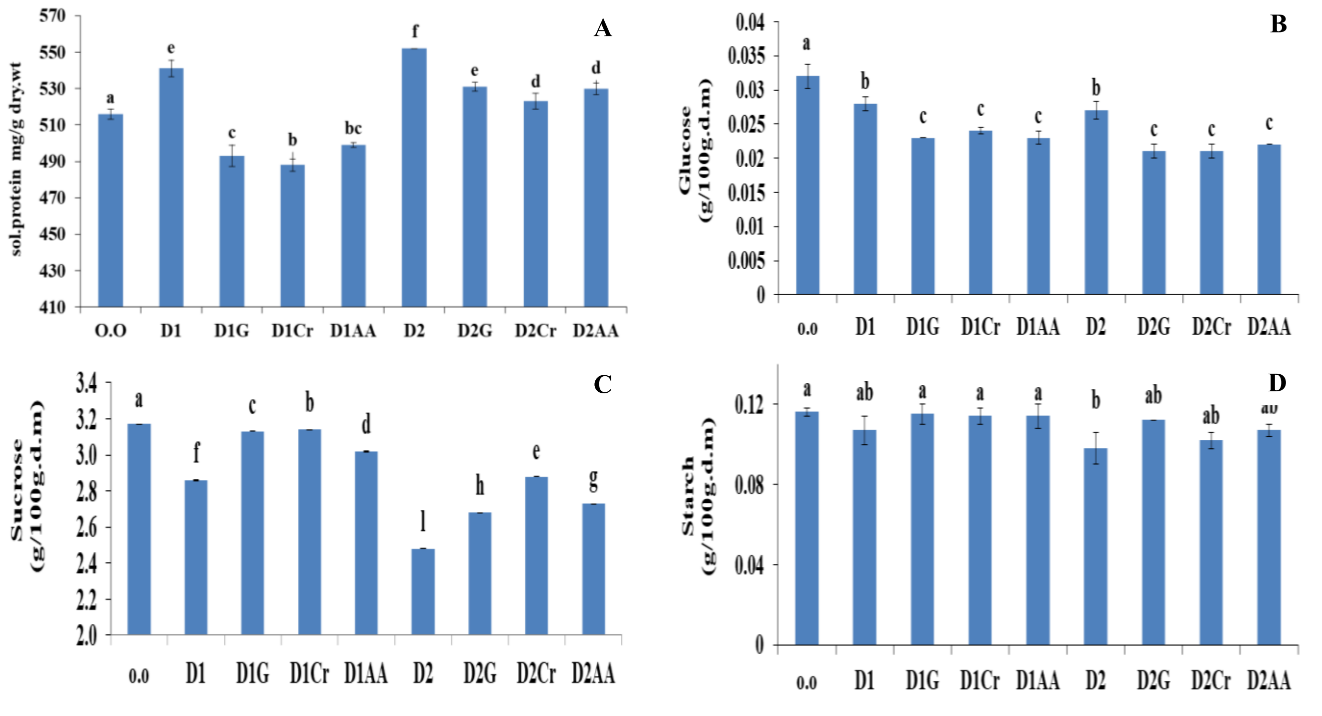

Fig. 2. Effect of drought stress by water withholding for 0.0 days (control); 14 days (D1), or 22 days (D2) on total soluble protein content (A), glucose (B), sucrose (C) and starch (D) of *Vicia faba* yielded seeds grown from presoaked seeds for 10 hrs in 100% garlic (G), 100 % carrot (Cr) extracts or 0.5 mM ascorbic acid (AA). Values are means of three replicates ± SD. Different letters indicate statistically significant differences ($p \leq 0.01$).

Table 1. Effect of drought stress by water withholding for 14 days (D1), or 22 days (D2) on categorization of molecular masses (MM) of protein bands as revealed by SDS-PAGE of protein extract of *Vicia faba* yielded seeds grown from presoaked seeds for 10 hrs in 100% garlic extract (G), 100% carrot (Cr) extract or 0.5 mM ascorbic acid (AA).

Stress level	M.M (kDa) of bands newly synthesized under stress	M.M (kDa) of bands present in control, disappeared under stress	M.M (kDa) of bands present in control, disappeared under stress, reappeared with priming			M.M (kDa) of bands newly synthesized with priming		
			Cr	G	AA	Cr	G	AA
D1	245, 124, 82, 58, 46, 36, 28, 15	218, 122, 85, 51, 37, 31, 16	85	16	37	282, 134, 64, 55, 30, 22, 17	259, 130, 53, 45, 35, 29, 19	306, 140, 95, 71, 59, 49, 34, 26, 20
D2	127, 79, 60, 50, 40, 33, 15, 13	218, 122, 67, 51, 37, 31, 16, 10	31	122, 37, 31	122, 37, 31	315, 124, 76, 63, 55, 49, 38, 21	22, 290	20, 290

Table 2. Effect of drought stress by water withholding for 22 days on the ISSR banding patterns on *Vicia faba* yielded seeds grown from presoaked seeds for 10 hrs in 100% garlic (G), 100% carrot (Cr) extracts or 0.5 mM ascorbic acid (AA).

Stress level	Molecular size (bp) of newly synthesized bands under D2, disappeared with seed priming	Molecular size (bp) of bands in control, disappeared under D2, and reappeared with seed priming	Molecular size (bp) of bands newly synthesized with priming (Cr, G or AA)
D2	461, 518, 544, 1540, 2174	466, 601, 658, 714, 841, 865, 1601	295, 301, 373,428, 491, 496, 582, 618, 641, 683, 706, 744, 825, 849, 969, 1041, 1615

ISSR-PCR analysis: The results of the ISSR banding patterns of DNA of the yielded seeds (Fig. 3 and Table 2) showed that drought stress resulted in the release of five new bands and the disappearance of seven others that were detected in control. On the other hand, seed presoaking in G, Cr extracts or AA indicated a significant amelioration of the harmful effects of drought stress, where they not only restored about 50% of the bands which disappeared under stress, but also resulted in the complete disappearance of the detected bands under D2 stress. The molecular sizes of these bands in bp are recorded in Table (2).

Discussion

Data of the current study showed that the two treatments of drought stress led to a highly significant decrease in the measured yield parameters. These results were in conformity with those obtained by Darkwa *et al.*, (2016) on *Vicia faba*. This reduction in yield parameters under drought stress may be because of decrease in leaf area, premature leaf senescence, oxidation of chloroplast lipids and alterations in the assembly of pigments and proteins, leading to lowering the supply of carbon assimilate, reduced photosynthetic rate and consequently minimal biomass production, as well as diminished translocation of assimilates towards the developing seeds (Abid *et al.*, 2016; Kasim *et al.*, 2017).

Despite the negative effect of drought on yield parameters, drought caused no change in both the number of pods/plant and number of seeds/pod. This phenomenon might indicate that drought negatively affected all yield criteria expressed in masses, which might be considered more important and affecting yield efficiency rather than the numbers of pods/plant and seeds/pod.

Plants subjected to drought stress and seed priming overcame the inhibitory effects of drought, where the % of mature seeds/pod, mass of pods/plant, mass of seeds/pod, mass of seeds/plant and mass of 1000 seeds were recovered and enhanced. These results are in agreement with those reported by Abdul Qados (2014) on soya beans *Glycine max* plants. The enhancement in yield parameters of the primed-drought stressed seeds may be due to the positive effect of garlic, carrot and ascorbic acid in the increase of leaf area, water content, growth, decrease in ROS production, scavenging oxidative agents of chloroplast lipids and changes in structure of pigments and proteins as recorded by Kasim *et al.,* (2017). This combination of effects leads to a better supply of carbon assimilate, healthy photosynthetic apparatus, higher photosynthetic rate and consequently, more biomass production and higher translocation of assimilates towards the produced seeds.

The present data revealed that the two treatments of drought stress resulted in a reduction in glucose, sucrose and starch contents of the yielded seeds. These declines may be due to that drought affects enzymes concerned with the mechanism of photosynthesis, in turn, inhibits the synthesis of carbohydrates (Gill *et al.,* 2011). Presoaking the seeds in garlic or carrot extracts or in ascorbic acid resulted in a significant decline in the glucose content of the yielded seeds, while sucrose and starch contents were risen. This was in accordance with the results of Kasim *et al.,* (2017) recorded in the leaves of the vegetative stage of vicia beans.

The total soluble protein content of the yielded seeds showed a highly significant increase with D1 and D2, which was similar to the trend previously obtained in the vegetative stage reported by Kasim *et al.,* (2017). This increase may be due to the induction of stress proteins, which are particularly involved in the maintenance of cell redox and might act as antioxidant enzymes (Shu *et al.,* 2012). On the other hand, in case of D1, seed priming with garlic, carrot extracts or ascorbic acid led to a reduction in total soluble protein content. This decline in protein content may be due to the suppression of transamination process, the increase of proteolysis as well as the reduction of protein synthesis (Baraka, 2008).

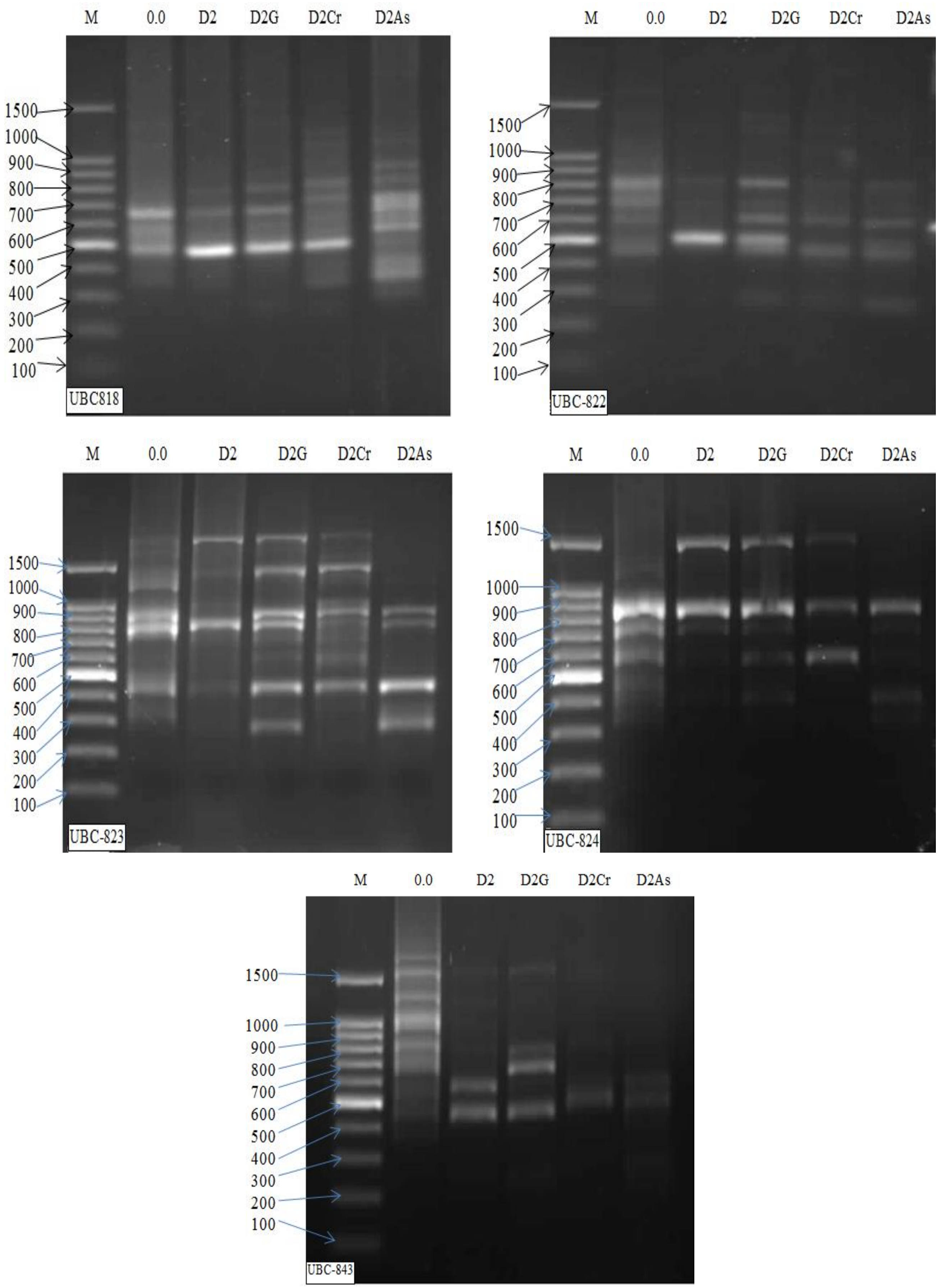

Fig. 3. Effect of drought stress by water withholding for 14 days (D1), or 22 days (D2) on ISSR analysis of *Vicia faba* yielded seeds grown from presoaked seeds for 10 hrs in 100% garlic (G), 100% carrot (Cr) extracts or 0.5 mM ascorbic acid (AA). (Using 5 primers). (UBC 818, UBC 822, UBC823, UBC 824 and UBC 843).

Six of the Heat Shock Protein families (HSPs) with MM ranging between 250 kDa and 15 kDa with roles to play in combating abiotic stresses in plants are represented in our study. Thus, the protein bands with MM 124 kDa and 127 kDa, may function as HSP 100 which play a crucial role in re-solubilizing protein aggregates via interactions with the sHSP chaperone system (Bosl *et al*., 2006). The protein band with MM 82 kDa, may function as HSP 90 and regulates protein folding and plays a key role in signal transduction networks, protein degradation and protein trafficking (Park & Seo, 2015). The protein band with MM 79 kDa, belongs to HSP 70 and acts as a chaperone for newly synthesized proteins to prohibit their accumulation as aggregates as well as to maintain proper protein folding through their transfer to their final location (Park & Seo, 2015). The protein bands with MM 58 kDa and 60 kDa, are members of the HSP 60 family which help in protein folding and subunit assembly (Efeoglu, 2009). The protein bands with MM 46 Da, 40 kDa and 36 kDa, of the HSP40 family are known to induce HSP70 affinity for clients (Kampinga & Craig, 2010). In addition, the protein bands with MM 28 kDa and 33 kDa represent the HSP30 family with their special mechanism of drought tolerance (Farooq *et al.,* 2008). The protein bands with MM 15 kDa operate as sHSP stopping undesired protein–protein interactions and assisting in refolding of denatured proteins (Gupta *et al.,* 2010).

Priming of *Vicia faba* seeds with G, Cr extracts or AA before sowing them and subjection to drought stress mitigated the mischievous effects of drought stress where the stress-protein bands were disappeared. This reflects the ability of seed priming in the enhancement of *Vicia faba* tolerance for drought stress. This enhancement was not achieved only by the disappearance of stress protein bands but also by the induction of some newly synthesized bands. The appearance of these newly synthesized bands may be due to the presence of abscisic acid in both extracts that plays a vital role in the synthesis of storage proteins (Tuteja, 2007). It may be suggested that the expression of stress-induced proteins is an adaptative strategy of plants to drought stress tolerance (Messaitfa *et al*., 2014).

Plant responses to drought stress are complicated and induce alterations at the molecular levels (Maraghni *et al*., 2014). The disappearance of some DNA bands with drought stress may reflect the damage caused by ROS at the genetic level. It may also be related to other processes such as DNA damage (e.g., single- and double- strand breaks, modified bases, oxidized bases, DNA protein cross-links), point mutations and/or chromosomal rearrangements induced by genotoxins (Aksakal, 2013). The appearance of new PCR products may disclose an alteration in some oligonucleotide priming sites due to mutations (new annealing events), large deletions and/or homologous recombinations (Atienzar & Jha, 2006).

Presoaking in carrot extract, garlic extract or ascorbic acid before their subjecting to drought stress indicated a considerable amelioration of the hurtful effects of drought stress, where they not only restored about 50% of the DNA bands which disappeared under stress, but also resulted in the complete disappearance of the detected bands under D2 stress. This may be due to the decrease of ROS formation, which disturbs the structure of nucleic acids. In addition, AA increases P and K uptake, which are necessary for the formation of DNA and RNA (Abdul Qados, 2014), taking into consideration that P, K and AA were also detected in the extracts of carrot and garlic as recorded by Kasim *et al.,* (2017).

Therefore, it seems advisable to recommend the use of an aqueous extract of either carrot roots or garlic cloves for not only mitigating the harmful influences of drought stress on the yield of *Vicia faba* but also for improving its quality with no discernible adverse effects. These two natural materials have the added advantages of being locally abundant, cheap, environmentally friendly and safe to human health.

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