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Protein Electrophoresis and DNA in Herbs Produced from Irradiated *Ambrosia maritima* Seeds Grown under Soil Salinity and Their Resistance to Insect

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ABSTRACT

Damsisa (*Ambrosia maritima* L.) is one of the wild plants present in Egypt and different African countries of the Nile Valley. It considered as potential source of molluscicides for treatment of infected sites. In this study, DNA amplifications technique and protein electrophoresis were used for the evaluation of response of Damsisa herbs to gamma rays (γ-rays), soil salinity and their interaction on alleviation of salt stress. This study also examined the effect of herb as bio-resistant for insect infestation in *Phaseolus* beans. Protein electrophoresis revealed that the number of protein bands separated from plants grown in saline soil not changed either grown from irradiated or un-irradiated seeds except 40 Gray (Gy) dose. Meanwhile, it was observed that mixing Damsisa herb with infested *Phaseolus* beans reduced insect ability to lays eggs or complete life cycle. Also, it was found that herbs produced from irradiated seeds and grown in normal or in saline soil were more effective in destruction of *Callosobruchus maculatus* insect and decreased the loss from infested beans.

Key words: *Ambrosia maritima*, γ-rays, soil salinity, protein electrophoresis, DNA, insect resistant

INTRODUCTION

Many plants had considered as potential source of molluscicides for treatments of infested sites. From these plants Damsisa (*Ambrosia maritima* L.) which is an annual herbaceous plant widely distributed throughout the Mediterranean region. Damsisa is considered as one of the wild plants present in the Nile valley of Egypt and in different African countries, its medicinal interest is due to its molluscicidal activities. The lethal effect of sesquiterpene lactones of the plant on snails (intermediate vectors *Schistosoma*) has been proven in Egypt (Sherif and El-Sawy, 1977; Abdel-Salam *et al*., 1984).

Salinity is one of the environmental factors which exert considerable alterations on plant growth and metabolism. The degree of these alterations depends on plant species, plant stage, as well as, salt levels. Seedling stage and floral development are often the most sensitive stages (Jones *et al*., 1989). Salinity can inhibits plants growth, affecting water absorption and biochemical processes as CO_2 and N_2 assimilation and protein synthesis (Delfine *et al*., 1999). Leblebici *et al*. (2011) observed that salt stress reduces water potential which causes ion imbalance or disturbances in ion homeostasis and this causes toxic effects. These effects alter the water status significantly and consequently lead to a reduction in the initial growth of the plant and limit its productivity. However, the direction and magnitude of these changes varied according to the level and duration of salinization treatment as well as the plant species used. In order to determine the tolerance of

plants to salinity stress, growth or survival of the plant measured. It integrates the up-or down-regulation of many physiological mechanisms occurring within the plant (Niknam and McComb, 2000). Many plants develop mechanisms either to exclude salt from their cells or to tolerate its presence within the cells (Leblebici *et al.*, 2011). The gamma rays (γ-ray) affect differentially the morphology, anatomy, biochemistry and physiology on plant depending on the irradiation level (Kim *et al.*, 2004; Kovacs and Keresztes, 2002; Wi *et al.*, 2005). The effects of γ-rays include change in cellular structure and metabolism of plant, e.g., dilution of thylakoid membranes, change in photosynthesis, modulation of antioxidant system and accumulation of phenolic compounds. This study used DNA amplifications technique and protein electrophoresis for the evaluation of response of Damsisa herbs to γ-rays, soil salinity and their interaction on alleviation of salt stress. Also, examine their effect as bio-resistant for insect infestation.

MATERIALS AND METHODS

Damsisa seeds (*Ambrosia maritima* L), were kindly provided by Horticulture Research Institute, Egyptian Ministry of Agriculture, Dokki, Egypt. Seeds were irradiated with different doses of gamma rays (0, 40 or 80 Gy). The irradiation process was carried out in the National Center for Radiation Research and Technology (NCRRT) using Cesium 137 as a source of γ-rays, the dose rate was 0.87 rad/sec. Seeds were sown in the first of October in fertilized soil according to the recommendations of the Egyptian Ministry of Agriculture and Land reclamation (150 kg calcium super phosphate/fed, 100 kg potassium sulphate/fed and 100 kg ammonium sulphate/fed) at 2008/2009 seasons. The pots, 30 cm in diameter were filled with 7 kg fertilized soil obtained from the farm of NCRRT. Salinity (4000 ppm) was obtained by adding the mixture of sodium chloride, calcium chloride and magnesium sulphate at the ratio of 2:2:1 by weight and mixed well with soil in each pot. The first group consists of un-irradiated control seeds and other two doses of irradiated seeds by 40 and 80 Gy, sown in normal soil (control). The first group was also serving as control for salinity. The second group as mentioned above in the first one was reply for salt treatments "4000 ppm". Ten seeds were germinated in pots filled with 0 and 4000 ppm soil and left to grew then plants were thinned to three plants per pot, all plastic pots were irrigated with tap water until field capacity. Samples were taken from treated and untreated plants, under normal soil and under the influence of soil salinity treatment. The produced plants were air dried and then milled for protein electrophoresis which performed according to the method previously described by Laemmli (1970) as modified by Studier (1973) and DNA which estimated by extraction and purification of samples using DN easy mini Kit (QIAGEN). Inter Simple Sequence Repeat (ISSR) procedure ISSR-PCR reactions were conducting using 5 primers. PCR was performed in 30 μL volume tubes according to Williams *et al.* (1990). The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94°C for 4 min followed by 45 cycles of 1 min at 94°C, 1 min at 57°C and 2 min at 72°C. The reaction was finally stored at 72°C for 10 min. The PCR products were separated on a 1.5% agarose gels and fragments sizes were estimated DNA ladder (1 kbp) mix was used as standard DNA with molecular weights of 1500, 10000, 8000, 6000, 5000, 4000, 3000, 200 and 100 bp. The run was performed for about 30 min at 80 V in mini submarine gel BioRad.

Insect experiment: This experiment aim to investigate the effect of hermetic storage of normal or irradiated herb of Damsisa when sprinkle infested *Phaseolus vulgaris* beans with *Callosobruchus maculates* insect. Infested beans and herb were incubated on 20/12/2011 and then count the number of alive or mortality insects after 2, 3 and 6 days (1st, 2nd and 3rd stage). This

experiment consists of three replicates; each replicate consists of {50 g seeds+0.1 g herb+25 insect} in 250 mL jars, all replicate were incubated at laboratory temperature ±28°C.

RESULTS AND DISCUSSION

SDS-electrophoretic protein pattern of *Ambrosia maritima* shoots produced from seeds irradiated with gamma rays and grown under natural or saline soil condition during 2008/2009 season were shown in Fig. 1 and Table 1. Herb produced from control seeds were exhibited twelve bands, decreased to eleven bands in herbs produced from seeds irradiated by 40 or 80 Gy and grew

Table 1: Protein pattern of *Ambrosia maritima* herbs produced from seeds irradiated with γ-rays and grown under natural or saline soil condition during 2008/2009 season.

		Natural soil			Saline soil (4000 ppm)		
		γ-rays (Gy)			γ-rays (Gy)		
Band No.	MW	Con	40	80	Con	40	80
1	105	1	1	1	1	1	1
2	88	1	0	1	1	1	1
3	77	1	1	0	1	1	1
4	70	1	1	1	1	1	1
5	40	1	1	1	1	0	1
6	34	1	1	1	1	1	1
7	30	1	1	1	1	0	1
8	25	1	1	1	1	1	1
9	21	1	1	1	1	1	1
10	19	1	1	1	1	1	1
11	12	1	1	1	1	1	1
12	11	1	1	1	1	1	1
Total		12	11	11	12	10	12

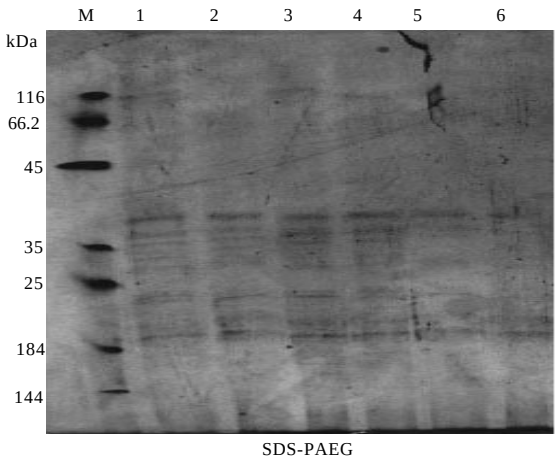

Fig. 1: SDS-PAGE profiles of protein pattern in *Ambrosia maritima* herbs as affected by γ-rays and soil salinity during 2008/2009 season. M: Molecular marker with different molecular weights. Lane 1, 2, 3: Control, 40 and 80 Gy grew in normal soil and Lane 4, 5, 6: Control, 40 and 80 Gy grew in "4000 ppm"soil

in normal soil. While in plants that grown in saline "4000 ppm" soil, the number of bands decreased to ten only in herbs produced from seeds irradiated by 40 Gy. But, the number of bands unchanged (12 bands) in case of unirradiated samples or those produced from irradiated seeds by 80 Gy. Thus, the twelve bands separated from un-irradiated herbs grown under saline soil (4000 ppm) equal to that grown in normal soil refer that Damsisa plants can tolerate soil salinity. The molecular weights ranged between 11-105 kDa, the band of 77 kDa which disappeared in case of herb produced from seeds irradiated by 80 Gy and grown in natural soil, reappeared in herb produced from those planted in saline soil ascertain that radiation can alleviate the harmful effect of salinity for improving plant growth in saline soil.

The names and sequences of five primers that produced highly reproducible and scorable bands that conducted using ISSR-PCR reaction were shown in (Table 2, Fig. 2). Polymorphism due to 40,

Table 2: ISSR-PCR DNA of five primers in *Ambrosia maritima* shoots produced from seeds irradiated with γ-ray and grown under natural or saline soil condition during 2008/2009. (-)Negative specific marker and (+) Positive Specific marker

			Normal soil				Saline soil "4000"ppm			
			γ-rays (Gy)				γ-rays (Gy)			
Primer No.	Bands No.	Molecular weight (kDa)	C	40	80	Polymorphism	C	40	80	Polymorphism
Primer 14 A	1	900	1	1	1	Monomorphic	1	1	1	Monomorphic
	2	800	1	1	1	Monomorphic	1	1	1	Monomorphic
	3	700	1	0	0	Unique +ve	0	1	1	Unique -ve
	4	580	1	0	0	Unique+ve	0	0	0	-
	5	500	1	1	1	Monomorphic	1	1	1	Monomorphic
	6	430	1	1	1	Monomorphic	1	0	0	Unique +ve
	7	400	0	1	1	Unique -ve	1	1	1	Monomorphic
	8	350	1	1	1	Monomorphic	1	1	1	Monomorphic
	9	280	1	1	0	Unique -ve	0	1	1	Unique -ve
Primer 44 B	1	900	0	1	1	Unique -ve	1	1	0	Unique -ve
	2	600	1	0	0	Unique +ve	1	1	0	Unique -ve
	3	540	1	1	1	Monomorphic	1	0	1	Unique -ve
	4	420	1	0	0	Unique+ve	0	0	0	-
	5	380	1	1	1	Monomorphic	1	1	1	Monomorphic
	6	360	1	1	1	Monomorphic	1	0	1	Unique -ve
	7	290	1	1	1	Monomorphic	1	1	1	Monomorphic
	8	270	1	1	1	Monomorphic	1	0	1	Unique -ve
	9	240	0	1	1	Unique -ve	1	0	1	Unique -ve
Primer HB 15	1	1500	1	0	1	Unique -ve	0	0	0	0
	2	1100	0	0	0	0	1	1	1	Monomorphic
	3	980	1	1	1	Monomorphic	1	1	1	Monomorphic
	4	920	1	1	1	Monomorphic	1	1	1	Monomorphic
	5	630	1	0	0	Unique +ve	0	0	1	Unique +ve
	6	570	1	1	1	Monomorphic	1	1	1	Monomorphic
	7	500	1	1	1	Monomorphic	1	0	0	Unique +ve
	8	420	0	0	0	-	0	1	1	Unique -ve
	9	350	1	0	1	Unique -ve	0	1	0	Unique +ve
	10	240	1	1	1	Monomorphic	1	1	1	Monomorphic
	11	180	0	0	0	0	1	0	0	Unique +ve
Primer HB 12	1	1500	1	0	0	Unique +ve	1	0	0	Unique +ve

Table 2: Continue

			Normal soil				Saline soil "4000"ppm			
			γ-rays (Gy)				γ-rays (Gy)			
Primer No.	Bands No.	Molecular weight (kDa)	C	40	80	Polymorphism	C	40	80	Polymorphism
	2	1150	1	1	1	Monomorphic	1	1	1	Monomorphic
	3	900	1	1	1	Monomorphic	1	0	0	Unique +ve
	4	700	1	1	1	Monomorphic	1	1	1	Monomorphic
	5	620	1	1	1	Monomorphic	1	1	1	Monomorphic
	6	500	1	1	1	Monomorphic	1	1	1	Monomorphic
	7	460	0	0	0	-	1	1	0	Unique -ve
	8	330	1	1	1	Monomorphic	1	1	1	Monomorphic
	9	270	1	1	1	Monomorphic	1	1	1	Monomorphic
	10	200	1	1	1	Monomorphic	1	1	1	Monomorphic
Primer HB 8	1	1200	0	0	1	Unique +ve	1	1	0	Unique -ve
	2	900	0	0	1	Unique +ve	1	0	0	Unique +ve
	3	750	0	1	1	Unique -ve	1	1	1	Monomorphic
	4	680	1	1	1	Monomorphic	1	1	1	Monomorphic
	5	500	1	1	1	Monomorphic	1	0	0	Unique +ve
	6	430	1	1	1	Monomorphic	1	1	1	Monomorphic
	7	300	1	1	1	Monomorphic	1	0	0	Unique +ve
	8	280	1	1	1	Monomorphic	1	1	1	Monomorphic
	9	260	0	0	0	-	1	0	0	Unique +ve

80 Gy treatments were present in Fig. 2 with primers op-14, op-B44, op-H 8, op-HB12, op-HB15, respectively The highest number of fragment (11) was amplified by the primers, op HB15 followed by, op HB12 (10) and the lowest (9) by primers 14A, 44B and HB8. Banding pattern for the five random primers were scored in Table 2 as present (1) or absent (0). The maximum number of polymorphic bands were (4) in case of op-14 and op-B44 and the minimum number was (1) in case of op-12 in normal set while in"4000 ppm" salinity set (6) in case of 44B and (3) in 14A, HB12. All primers amplified DNA fragments from Damsisa DNA samples successfully. In primer 44B, the polymorphic band 420 pb represent +ve marker for Damsisa plants as it appeared in control sample but disappeared in irradiated 40 or 80 Gy samples. The opposite take place in primer 14A, when samples grown in 4000 ppm saline soil where control disappeared and 40, 80 Gy appeared. Also, at primer 14A, MW 400 bp appeared in all samples grown in normal or saline soil, except untreated samples. At HB12 primer, MW 900 bp considered negative marker for salinity as it disappeared from treatments (40 and 80 Gy) but +ve in control treated by 4000 ppm and in control set. The same observation was obtained by Hamideldin and Hussein (2009) on wheat, the changes in DNA profile was rare and may attribute to the difference in plant individual or to gamma rays. The percentage of polymorphism was 34.8% in normal soil and 48.8% in saline soil. Gurudeeban *et al.* (2011) concluded that the optimized isolation protocol of DNA and RAPD markers considered an efficient tools for molecular studies of salt marsh plants.

The effect of Damsisa herb on hatched larvae emerged from infested seeds and full grown insects deposit eggs and the produced larvae were fed on seeds. The number of alive, mortality insects and the shortage in seeds weight were recorded (Fig. 3). Compares values across column and standard error, it was observed that numbers of killed insects in the first day of count were the highest. The herb produced from irradiated seeds by 80 Gy had the highest mortality percentage (34.9%). After six days, the activity of herb on killing insects decreased in control herb to 23.8% and

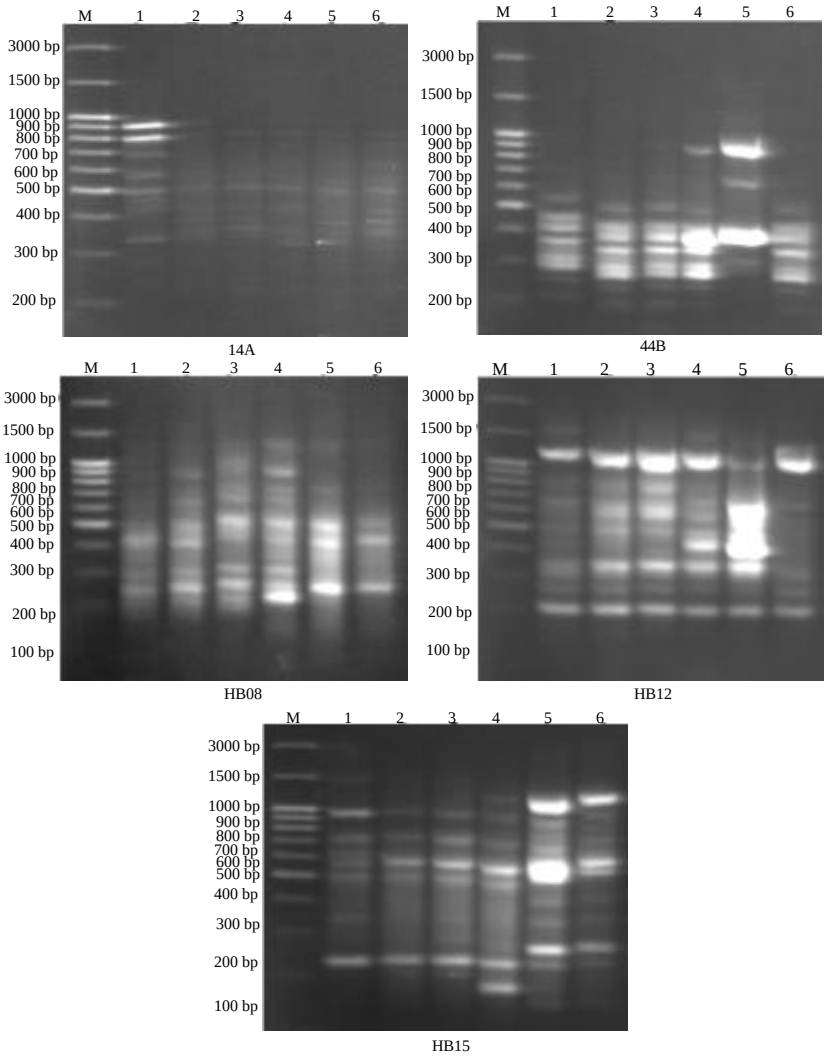

Fig. 2: ISSR-PCR DNA of *Ambrosia maritima* shoots produced from seeds irradiated with γ-rays and grown under natural or saline soil condition during 2008/2009 seasons

in irradiated herb to 29.1%. The alive insects' numbers ranged, in control herb after six days from beginning of experiment, between 69.7-76.8%. While in herb produced from 80 Gy treatment ranged from 65.2-70.9%. The rate of insects generation in control herb increase by 7.1% while in irradiated herb increase by 5.7% during six days of experiment. This refer that Damsisa herb were effective in reducing insect reproduction but the irradiated herbs were more effective in this respect. Concerning shortage in seeds weight in control herb during first stage, it was observed that the shortage in seeds weight sprayed by irradiated herb were less than those sprayed by control herb as shown in Fig. 3.

Synthetic pesticides are highly discouraged because of their adverse effect on human beings and environment. So, the use of hermetic storage of Damsisa herb in combination with infested *Phaseolus* beans for reduction emergence of the insect and its ability to lays eggs or complete life

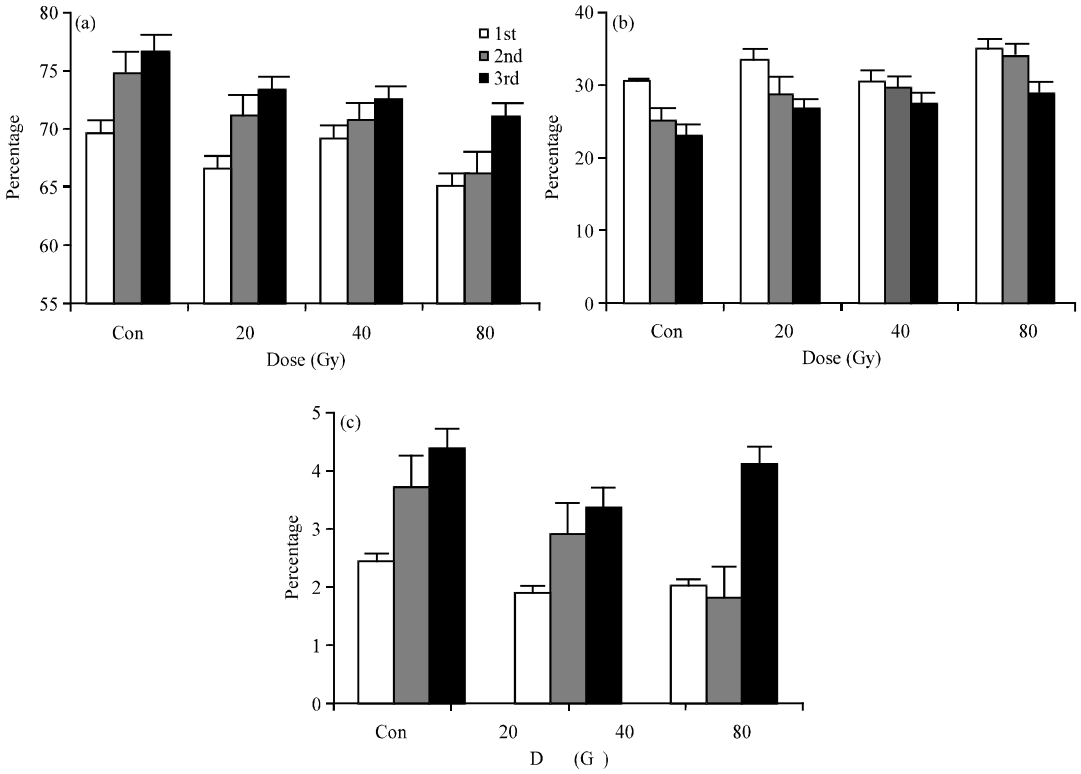

Fig. 3(a-c): Percentage of (a) Alive or, (b) Mortality insects and (c) Loss in beans weight as affected by Damsisa herbs grown in normal soil from control or γ-irradiated seeds during 2008/2009, 1st: 2 days, 2nd: 3 days and 3rd: 6 days

cycle. It was noticed that herbs produced from irradiated seeds grown in normal or in saline soil was more effective in increasing insect mortality and decreasing the loss from infested seeds. The recommended dose of Damsisa herb was 2 g kg^{-1} grains; it can use as one of natural component for reduce the application of the synthetic pesticides to control pests. Efficacy of various plant products have been reported by several authors against the stored grain pests (Bright *et al*., 2001; Boeke *et al*., 2004; Pugazhvendon *et al*., 2009).

CONCLUSION

The number of protein bands separated from plants grown in saline soil not changed either produced from irradiated or un-irradiated seeds. Damsisa plant can play a role in protection of stored grains from pest infestation and considered as biopesticides against *Callosobruchus maculates*. Many entomological studies needed to examine other pests on different storage crops for ascertain the effect of Damsisa herb as biopesticides.

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