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GENETIC EVALUATION AND RAPD MOLECULAR MARKERS FOR SALT TOLERANCE OF NEW PROMISING FABA BEAN LINES (*VICIA FABA* L.)

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ABSTRACT

Genetic evaluation was performed on ten Faba bean genoypes (*Vicia faba* L.) for salt tolerance. Salt tolerance related criteria, used in this study, were seed germination, plant height, osmotic pressure at 30 days and 60 days and proline content at 60 days. The obtained results confirmed more variabilities between new genotypes and varities under study. The LP7 new promising line was the most salt tolerant than other genotypes, while, Giza 714 considered the most salt sensitive in all studied criteria. Osmotic pressure at 30 , 60 days and proline content recorded the highest values in LP7 and subsequently decreased in the other tested genotypes. Osmotic pressure and proline content increased with increasing of salt concentrations, especially in the most tolerant line (LP7). However, the susceptible genotypes exhibited low value of osmotic pressure and proline content. The results showed also that a positive correlation had been found between seed germination % and plant height at 30 days, osmotic pressure at 30 days and proline content under 5000 ppm. Similarly, a positive and significant correlation was found between osmotic pressure at 30 days under 5000 ppm saline and both of osmotic pressure at 60 days and proline content under 5000 ppm.

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Accordingly, LP7 as a tolerant line and G714 as a sensitive cultivar were crossed for study of molecular markers related to salt tolerance by using bulked segregant analysis (BSA). Bulk of the two extremely F_2 plant (most tolerant and most sensitive F_2 groups) and two parents were used to develop some molecular genetic markers associated with salt tolerance in faba bean by using eleven RAPD primers. The RAPD-PCR products exihibited seven positive molecular markers in the tolerant parent with molecular sizes 366 bp for primer B16, 2406pb for primer B17, 583 bp for primer 320, 662 and 579 bp for primer F01, 342bp for primer F04 and 2361pb for primer F09. There were also eight negative molecular marker in the sensitive parent with molecular sizes 770 and 453bp for primer B17, 1433 and 975 bp for primer B20, 252bp for primer F05, 2408 and 768 bp for primer F06 and 225 bp for primer F08. These results showed the possibility of genotypes for salt tolerance in breading faba bean using molecular markers.

Keywords: Faba bean, salt stress, salt related traits, molecular markers, RAPD-PCR, bulked segregant analysis (BSA).

INTRODUCTION

Faba bean (*Vicia faba L.*) is the most important legume crop grown in Egypt. It is used in daily diets, especially, for lower income people. This is due to the high nutritive value of seeds, which contain about 30% protein (Atia *et al.*, 1995). Therefore, the breeders in Egypt and other developing countries have tried to improve yield and seed cocking quality (Kumari, 1996; Rabie *et al.*, 1996 and Omar *et al.*, 1999).

Salinity of the soil either natural or caused by irrigation in aried environment and excessive use of fertilizers is a great problem in agriculture. Grain legumes have multiple functions in traditional farming systems. They play an important role in the maintenance of soil fertility, particularly in dry rainfed areas. Pluse crops are generally considered to be more sensitive to subsoil constraints than cereal crops and farmers consider them unreliable in regions such as the southern Mallee of Victoria where sub-soil salinity is widespread. Alleviating such abiotic stresses through soil modification is not an economical or practical solution (Materne *et al.*, 2007).

Several faba bean varieties were screend for their response to high NaCl level to identify tolerant genotypes. The used in genetic evaluation of faba bean genotypes for salt tolerance are seed germination, plant height, osmotic pressure and proline content (Fukuta *et al.,* 2004 and Boddi *et al.*, 2010).

In Egypt, faba bean is a very important crop because it is cultivated in newly reclaimated lands, which suffering from salinity as a main problem. The local varieties were found to be sensitive to salinity and the productivity of these varieties is lower under salinity. A set salt tolerance of mutant lines were obtained in a study of desirable mutations induction faba bean under soil salinity (Soliman *et al.,* 2005). These lines are used in the present study.

Molecular markers developed by analysis of protein, isozymes, randomly amplified polymorphic DNA (RAPD), SSR and ISSR were used in different crops by many authors, i.e, Paran *et al.,* (1991); Abdel-Tawab *et al.* (2002) and Khan *et al.* (2002).

Bulked segregant analysis of F_2 plants as a simpler alternative to isogenic line analysis was developed by Michelmore *et al.* (1991), where each of the highest and lowest extremes of the F_2 population is bulked for the development of RAPD molecular markers. This method was applied in different crops (Avila *et al.,* 2003; Abdel-Bary *et al.,* 2005; Rashed *et al.*, 2006 and Torres *et al.,* 2008).

Therefore the aim of the present study is to evaluate some promising mutant lines of faba bean for salt tolerance and a trial was made to identify RAPD molecular markers associated with salt tolerance using bulked segregant analysis (BSA).

MATERIALS AND METHODS

Genetic Evaluation of Faba bean Genotypes under Salinity Conditions

The material used were 4 cultivars and 43 promising mutant lines. These genotypes were supplied by Dr. S. Soliman, Genetics Department, Faculty of Agriculture, Zagazig University (Soliman *et al.,* 2005). The cultivars and mutant lines were sown and selfed in season 2005. In the following season, 2006 these genotypes were planted under salinity conditions for screening their respone to Nacl salinity stress and to detect the critical toxic dose. On the basis of the screening, 2 cultivars and 8 mutant lines were chosen, name and characterization of these genotypes are given in Table (1).

In growing season of 2007, selfed seeds of the ten genotypes were grown in plastic bags, filled with 5 Kg of clay soil. The bags were arranged on complete randomized block design experiment having three Nacl saline treatments and with three replicates for each one.

Nacl concentration were 0 (as control), 4000 and 5000 ppm, and added to soil before sowing. Each replicate contained one bag for each genotype pretreatment having four seeds, in each genotype was represented by 12 plant in each treatment.

The investigation was carried out at the experimental farm and Green house of Genetic Department, Faculty of Agriculture, Zagazig University, Egypt.

The following characters were estimated:

1. Seed germination % after 7 days.
2. Plant height in cm after 30 days from planting.

Samples of leaves were taken for Laboratory procedures to determine osmotic pressure and proline concentrations:

1. The Osmotic pressure was estimated by transforming the total soluble solides to osmotic pressure, as air pressure (bar), after multiplying by 1.013 (Morgan, 1977). The total soluble solids were determined as refraction index using Zeiss refractometer. Osmotic pressure was measured in samples at 30 and 60 days old seedlings.
2. Determination of proline:

Proline concentration was determind chromatography according the method given by Bates *et al.* (1973). Fresh leaves samples of 30 old days seedlings of known weight were homogenized in 3% aqueous salfo salysilic acid and filtered. The filtrate was reacted with ninhydrin and glacial acetic acid for one hour at 100°C and proline was extracted with toluene on chromatography papers. The chromophoros containing toluene were airing dried and the eluted spots were spectrophotometrically measured at 520 nm.

Statistical Analysis

Collected data were analyzed using statiscal software SPSS version 9.0 Two-way analysis of Variance (ANOVA) was used to determine differences among genotypes. Relationships between variable characters were estimated as correlation coefficient. Heritability in broad sense was estimated as follows:

$$h^2(\text{in broad sense}) = \frac{\text{genotypic variance}}{\text{Phenotypic variance}} \times 100$$

Bulked Segregant Analysis (BSA)

Materials

Two genotypes of faba bean namely; LP_7 as (salt tolerance) and Giza 714 as (salt sensitive) were chosen after evaluation for salt tolerance.

Sand culture experiment

The self seed of both genotypes (LP_7 and G_{714}) were grown in field and crossed to obtained the F_1 seeds in season 2007. The F_1 seeds were grown in the field and selfed to obtain the F_2 seeds in season 2008.

Seeds of the two parents and F_2 generation were sown in sand culture in pots. The seedling were irrigated with the base nutrient solution every three days until day 14 from sowing (pre-treatment) then; salinity treatment (4000 ppm) was conducted at 30 days from sowing (post-treatment). Samples of the two parents and from F_2 plants, which were represented by 65 plants, were taken for molecular analysis at 60 days (the end of the experiment). The five most salt tolerance and the five most sensitive F_2 plants were selected according to their response to salinity and used for bulked segregant analysis as shown in Table (3).

Molecular DNA Analysis

Genomic DNA extraction

DNeasy™ plant Mini Kit (Qiagen Inc., Cat. No 69104) was used for DNA isolation as described in the manufacturer manual from plant samples (the two parents and two extreme group of F_2 plants) using bulked segregant analysis (BSA) technique.

RAPD-PCR

PCR reactions were performed according to Williams *et al.* (1990) using eleven 10-mer primers (Table 2).

Amplification was performed on a top quality thermel cycler programmed for 45 cycles of 1 minute at 94°, 1 minute at 36° and 2 minute at 72°. Amplification products were analyzed by electrophoresis in 1.4% agarose gels and detected by staining with ethidium bromide.

Table 1. Name, Source and characterization of *faba bean* genotypes

Name	Source	Characterization
1. G_{714}	Cross between (83/908/0462x503/453/83)	Moderately sensitive to salinity
2. Improved Giza-$_3$	Cross between Giza/Xintroduced Dutch (NA29). 1978	Moderately tolerance to Salinity
3. LP_7	New developed strain*	Long pod mutant line from improved Giza 3
4. EF_2	New developed strain*	Early flowering mutant line from improved Giza 3
5. SS_{15}	New developed strain*	Small seed mutant line from Giza 714
6. $H.N.S_6$	New developed strain*	High number of seed mutant line from Giza 2
7. $L.P_5$	New developed strain*	Long pod mutant line from improved Giza 3
8. $S.S_{17}$	New developed strain*	Small seed mutant line from Giza 716
9. L.F. P_6	New developed strain*	Low height of first pod mutant line from Giza 714
10. D_5	New developed strain*	Dwarf mutant line from improved Giza 3

* By Soliman *et al.* (2005) Genetics Dept., Zagazig Univ.

Table 2. List of RAPD primers (Operon Technology USA)

No.	Primer	Sequence(5' to 3')
1	A07	5'-GAAACGGGTG-3'
2	B14	5'-TCCGCTCTGG-3'
3	B16	5'-TTTGCCCGGA-3'
4	B17	5'-AGGGAACGAG-3'
5	B20	5'-GGACCCTTAC-3'
6	F1	5'-ACGGATCCTG-3'
7	F4	5'-GGTGATCAGG-3'
8	F5	5'-CCGAATTCCC-3'
9	F6	5'-GGGAATTCGG-3'
10	F8	5'-GGGATATCGG-3'
11	F9	5'-CCAAGCTTCC-3'

RESULTS AND DISCUSSION

Genetic Evaluation of Faba bean Genotypes for Salt Tolerance

Significant difference between studied genotypes were recorded for most studied saline related traits and saline concentration as well as genotypes saline interactions. Also moderate to high heritability values in broad sense were recorded (Table 3). These results might suggest that genetic improvement could be gained for salt tolerance related characters (Avila *et al.,* 2007; Arbaoui *et al.,* 2008 and Mafakheri *et al.,* 2010).

The results in Table 4 showed the mean performance of the studied salinity related traits and indicated that the highest values of seed Germination and plant height under salinity were recorded for line LP_7, while the lowest was obtained in line $S.S_{17}$ and cultivars G_{714}. In Osmotic pressure at 30 days, the result indicated that the line LP_7 had the highest value compared with all the other lines, Also the same tend was observed at 60 days. These observations were clearly detected under 5000 ppm saline concentration. The result of proline content indicated that, the highest value was scored for LP_7 line, while the cultivars G_{714} was the least.

Correlation coefficient (r) for ten characters (five under normal condition (0 ppm) and the same five characters under salt stress condition (5000ppm) are shown in (Table 5). The results confirmed no correlation between five criteria, i.e., seed germination%, plant height at 30 days, osmotic pressure at 30 days, osmotic pressure at 60 days and proline content under normal condition (0 ppm salt stress).

Highly significant and positive correlation were recorded for seed germination % and plant height at 30 days, osmotic pressure at 30 days and proline content (Table 6). These results confirmed the selected related criteria under study are more suitable for discovery of salt tolerance genotypes. Moreover, osmotic pressure at 30 days were highly correlation with osmotic pressure at 60 days and proline content under salt stress. These result confirmed that evaluation at any faba bean genotypes for salt tolerance should be carried out under salinity stress. These results agreed with Neeraj-Gupta *et al.* (2006).

Ranking genotypes were estimated as flows under salt stress condition (5000 ppm). The genotypes which possess high value for each trait obtained on 10 point and sequencing of all genotypes per each character and the basis of the value for each (Table 7). The ranking pattern of the genotype revealed that the line LP_7 had the highest score and eventually it ranked the first. Therefore, it could be consedered the most salt tolerante line. While, the genotype G_{714} ranked least in this suspect and showed its sensitiveness. Carol *et al.* (2002); Ashraf and Foolad (2005) and Mafakheri *et al.* (2010) obtained similar results concerning salt tolerance related characters.

Molecular Genetic Marker

RAPD molecular analysis for salt tolerance

Out of a total of 65 plants of F_2 which were arranged in descending order according to their overall performance for five different related characters traits under salt stress, the top and the lowest five F_2 plants were chosen for subsequent bulking segregant (Table 8).

DNA was isolated from the two contrasting parents, LP_7 as a salinity tolerant parent and G_{714} as a salinity sensitive one, and DNA bulks of tolerant and sensitive groups of F_2 population segregating for their response to salt stress. These genotypes were tested against fourteen mer random primers.

All RAPD primers (A07, B14, B16, B17, B20, F01, F04, F05, F06, F08 and F09) were found to have amplified PCR products. Only, nine primers gave polymorphism, which can be used in developing molecular markers for salinity tolerance. These bands are shown in Fig. 1 and summarized in Table 9. The PCR products exhibited seven positive molecular markers found only in the tolerant parent (LP7) and the tolerant F_2 bulk with molecular sizes 366, 2406, 583, (662, 579), 342 and 2361 bp for primers B16, B17, B20, F01, F04 and F09, respectively. While these bands were absent in sensitive parent and the sensitive F_2 bulk. There were also eight negative molecular markers which were exhibited in the sensitive parent G714 and the sensitive F_2 bulk with molecular sizes of (770, 453), (1433, 975), 252 , (2408, 768) and 225 bp using primers B17, B20, F05, F06 and F08, respectively.

So, these results indicated that these fifteen positive and negative RAPD markers could be considered as reliable markers for salinity tolerance in Faba bean (*Vicia faba* L.). Abo Def *et al.* (2005) and Afiah *et al.* (2007) found the similar results.

Table 3. The mean squares of five salt tolerance related criteria and heritability in ten faba bean genotypes

S.O.V	Df	Seed Germination 7 days %	Plant height at 30 days	Osmotic pressure at 30 days	Osmotic pressure at 60 days	Proline content
		Mean squares				
Replication	2	21.111	2.734	0.998	1.089	4.838
Salt treatments (a)	2	26083.611**	211.861**	59.837**	66.984**	37.608**
Lines (b)	9	734.444	8.497*	2.849**	9.897**	28.789**
a x b	18	366.944	3.767*	1.323*	1.516**	13.020**
Error	58	508.755	2.330	0.110	0.176	1.295
		Genetic parameters				
h^2	-	-	30.126	83.916	88.674	81.382

H^2_{bs} = Broad sense heritability. * Significant at (5%). ** Highly significant at (1%)

Table 4. Mean performance ($\overline{X}$) and least significant difference (LSD) of five studied salt tolerance related traits in the ten faba genotypes under three Nacl saline treatments

Lines	Seed Germination %			Plant height at 30 days			Osmotic pressure at 30 days		
	Con.	4000ppm	5000ppm	Con.	4000ppm	5000ppm	Con.	4000ppm	5000ppm
1. G_{714}	100.00	38.333	21.333	14.500	11.500	11.167	4.221	5.754	6.611
2. G_3	86.667	53.333	21.667	16.333	12.000	10.833	4.221	5.234	6.747
3. LP_7	93.333	63.333	46.667	16.333	14.000	13.333	5.403	7.247	8.936
4. EF_2	55.00	46.667	21.667	14.900	11.833	10.167	4.728	7.267	7.780
5. $S.S._{15}$	78.333	21.667	21.667	14.833	11.333	9.000	5.065	6.747	7.598
6. $H.N.S_6$	86.667	46.667	30.000	15.500	12.667	11.500	5.403	6.754	7.247
7. LP_5	86.667	55.00	30.333	15.667	12.167	8.833	5.403	7.257	7.935
8. $S.S._{17}$	78.330	55.00	5.000	17.500	10.167	8.000	5.403	5.910	7.085
9. $L.F.P_6$	71.667	21.667	21.667	16.166	13.000	10.333	3.715	5.741	7.767
10. D_5	86.667	46.667	21.667	12.667	11.833	8.833	3.883	6.585	7.767
Average	82.333	44.833	24.166	15.440	12.050	10.200	4.745	6.449	7.547

LSD	5%	1%	5%	1%	5%	1%
Salt treatments (a)	0.171	0.227	0.216	0.288	0.587	0.781
Lines (b)	0.312	0.415	0.395	0.526	1.072	1.426
(a x b)	0.542	0.721	0.686	0.913	1.860	2.475

Table 4. Continued

Lines	Osmotic pressure at 60 days			Proline content		
	Con.	4000ppm	5000ppm	Con.	4000ppm	5000ppm
1. G_{714}	6.598	7.286	7.818	3.650	3.803	4.397
2. G_3	5.909	6.585	7.922	4.963	5.233	7.657
3. LP_7	7.078	8.585	11.760	7.597	8.553	12.197
4. EF_2	6.922	8.273	10.468	4.717	4.740	6.240
5. $S.S._{15}$	6.922	8.779	10.468	4.330	4.440	6.180
6. $H.N.S_6$	6.585	9.286	9.637	4.043	4.280	5.680
7. LP_5	7.091	8.273	10.312	5.997	6.160	8.640
8. $S.S._{17}$	7.429	8.780	9.793	4.467	4.653	5.657
9. $L.F.P_6$	7.429	9.286	9.611	5.807	5.967	7.681
10. D_5	6.585	9.286	10.643	4.947	5.080	6.681
Average	6.855	8.442	9.843	5.052	5.291	7.101
LSD	5%	1%		5%	1%	
Salt treatments (a)	0.216	0.288		0.587	0.781	
Lines (b)	0.395	0.526		1.072	1.426	
(a x b)	0.686	0.913		1.860	2.475	

Table 5. Correlation coefficient (r) among salt tolerance related criteria under normal conditions

	Trait	Under 0 ppm saline				
		Seed Germination %	Plant height at 30 days	Osmotic pressure at 30 days	Osmotic pressure at 60 days	Proline content
Under 0 ppm saline	Seed Germination %	1.00				
	Plant height at 30 days	-0.085	1.00			
	Osmotic pressure at 30 days	0.670	0.444	1.00		
	Osmotic pressure at 60 days	-0.350	0.355	0.281	1.00	
	Proline content	0.065	0.287	0.156	0.333	1.00

Table 6. Correlation coefficient (r) among salt tolerance related criteria under 5000 ppm salt conditions

	Trait	Under 5000 ppm saline				
		Seed Germination %	Plant height at 30 days	Osmotic pressure at 30 days	Osmotic pressure at 60 days	Proline content
Under 5000 ppm saline	Seed Germination %	1.00				
	Plant height at 30 days	0.757*	1.00			
	Osmotic pressure at 30 days	0.690*	0.278	1.00		
	Osmotic pressure at 60 days	0.455	0.008	0.911**	1.00	
	Proline content	0.755*	0.473	0.814**	0.579	1.00

* Significant at (5%). ** Highly significant at (1%)

Table 7. Ranking of the faba genotypes over all the five investigated characters related traits

Lines	Seed Germination %	Plant height at 30 days	Osmotic pressure at 30 days	Osmotic pressure at 60 days	Proline content	Mean
G_{714}	2	8	1	1	1	13
G_3	3	7	2	2	7	21
LP_7	10	10	10	10	10	50
EF_2	4	5	8	7	5	29
$S.S._{15}$	5	4	5	7	4	25
$H.N.S._6$	8	9	4	4	3	28
LP_5	9	3	9	6	9	36
$S.S._{17}$	1	1	7	5	2	16
$L.F.P_6$	6	6	3	3	8	26
D_5	7	2	6	9	6	30

Table 8. The most tolerant and the most sensitive F_2 plant according to their performance in some salt tolerance related traits

	Plant height	Osmotic pressure at 30 day	Osmotic pressure at 60 days	Proline content
Most tolerant F_2 plants	24	7.931	10.121	11.5
	26	8.312	9.722	11.5
	26	8.513	11.433	10.5
	24	8.211	11.040	12.0
	26	8.910	11.501	11.0
Most sensitive F_2 plants	16	5.547	7.311	4.5
	18	5.234	7.521	5.0
	16	5.910	7.020	4.5
	18	5.403	5.615	4.5
	18	6.511	6.312	4

Table 9. RAPD marker for salt tolerance in the studied population

Primer name	PBN	M.S (bp)	TP	SP	F_2Tb	F_2Sb	MT
B16	8	366	1	0	1	0	Positive
B17	1	2406	1	0	1	0	Positive
	10	770	0	1	0	1	Negative
	15	453	0	1	0	1	Negative
B20	2	1433	0	1	0	1	Negative
	4	975	0	1	0	1	Negative
	7	583	1	0	1	0	Positive
F01	4	662	1	0	1	0	Positive
	6	579	1	0	1	0	Positive
F04	6	342	1	0	1	0	Positive
F05	5	252	0	1	0	1	Negative
F06	1	2408	0	1	0	1	Negative
	7	768	0	1	0	1	Negative
F08	5	225	0	1	0	1	Negative
F09	1	2361	1	0	1	0	Positive

TP = tolerant parent SP = sensitive parent F_2Tb = tolerant F_2 bulk F_2Sb = sensitive F_2 bulk

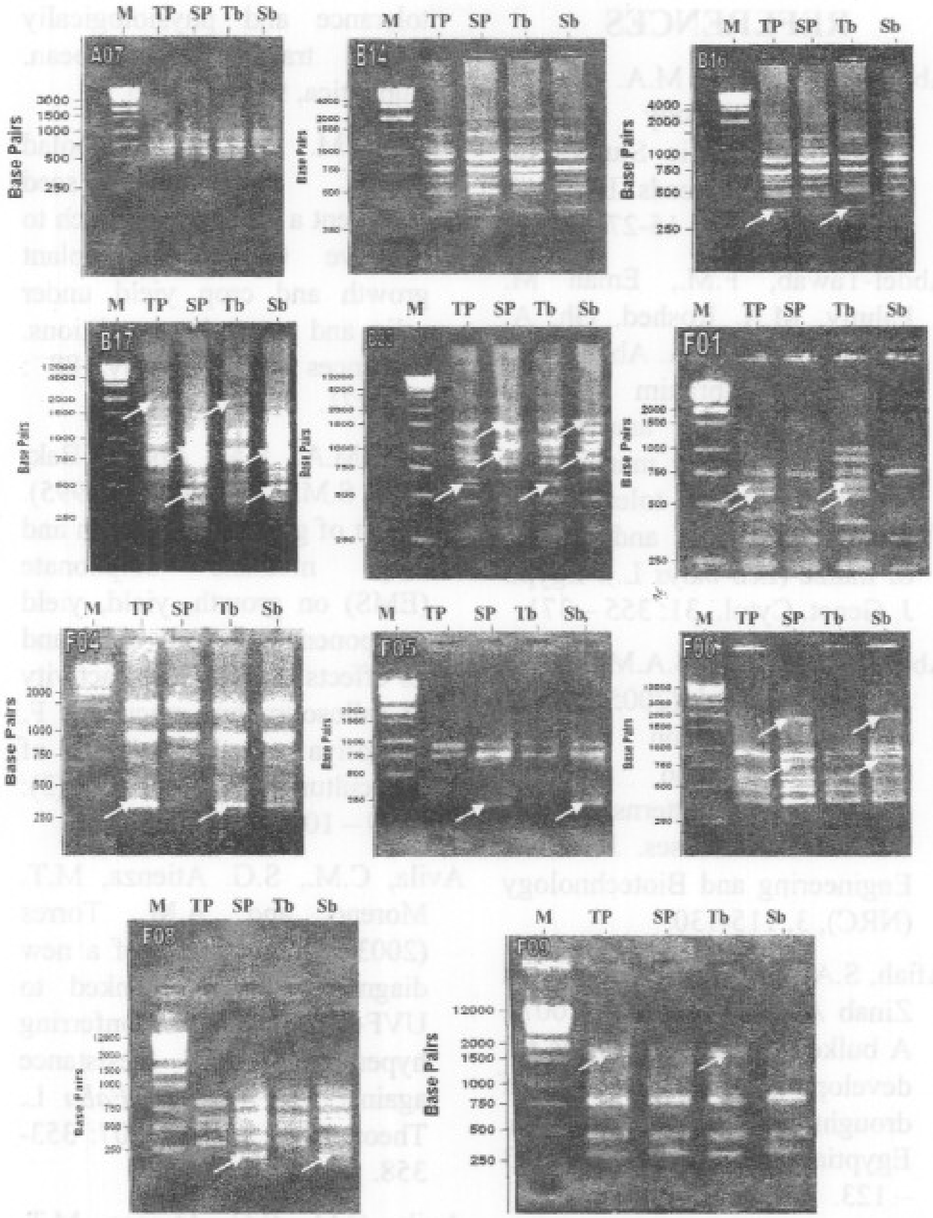

Fig. 1. RAPD-PCR banding patterns for eleven primers (A07, B14, B16, B17, B20, F01, F04, F05, F06, F08 and F09) with Faba bean genotypes under salinity stress

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التقييم الوراثى والمعلمات الجزيئية لتحمل الملوحة فى سلالات مبشرة من الفول البلدى

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أجرى تقييم وراثى لعشرة تراكيب وراثية من الفول البلدى تحت تأثير الملوحة وذلك لاكتشاف التباين بينها فى مدى استجابتها لتحمل الملوحة. هناك بعض الصفات التى لها علاقة بصفة تحمل الملوحة مثل نسبة الإنبات وطول النبات وهذه تعتبر صفات مورفولوجية معلمة بالنسبة تحمل الملوحة، أما الصفات الأخرى المستخدمة فهى الضغط الإسموزى ومحتوى الحمض الأمينى البرولين. ولقد تراوحت قيمة المكافئ الوراثى لهذه الصفات من المتوسطة إلى المرتفعة لذلك لابد من إجراء تحسين وراثى لمثل هذه السلالات المستخدمة موضع الدراسة. تم تقدير معامل الارتباط لخمس صفات والتى أوضحت معنوية عالية وارتباط إيجابى بين نسبة الإنبات وكل من: طول النبات عند ٣٠ يوم والضغط الإسموزى عند ٣٠ يوم ومحتوى البرولين عند تركيز ٥٠٠٠ ppm ملوحة. وكذلك وجد ارتباط عالى المعنوية وموجب بين الضغط الإسموزى عند ٣٠ يوم تحت تركيز ٥٠٠٠ppm ملوحة وكل من الضغط الإسموزى عند ٦٠ يوم ومحتوى البرولين تحت نفس التركيز.

استخدمت السلالة LP_7 كسلالة مقاومة للملوحة فى التهجين مع الصنف G714 كصنف حساس للملوحة وتم الحصول على بذور الجيل الأول والتى تركت للتلقيح الذاتى وذلك للحصول على بذور الجيل الثانى والتى استخدمت مع الآباء للتعرف على المعلمات الجزيئية التى لها علاقة بمقاومة الملوحة وذلك من خلال استخدام تحليل (BSA)، حيث أجرى تقييم لكلاً من الآباء المنتخبة ونباتات الجيل الثانى لبعض الصفات التى لها علاقة بمقاومة الملوحة والتى من خلالها قسمت نباتات الجيل الثانى إلى مجموعتين أحدهما نباتات مقاومة للملوحة والأخرى نباتات حساسة للملوحة. استخدمت الآباء المنتخبة وكلاً من المجموعتين التحمل والحساسة للتعرف على بعض المعلمات الجزيئية التى لها علاقة بمقاومة الملوحة وذلك باستخدام ١٤ بادئ. أظهر التحليل (BSA) أن هناك ٧ معلمات جزيئية موجبة وموجودة فى الأب المقاوم ذات أحجام ٣٦٦ زوج قواعد للبادئ B16 و ٢٤٠٦ زوج قواعد للبادئ B17 و ٥٨٣ زوج قواعد للبادئ B20 و(٥٧٩ ، ٦٢٢) زوج قواعد للبادئ F01 و ٣٤٢ زوج قواعد للبادئ F04 وأخيراً ٢٣٦١ زوج قواعد للبادئ F09 كما أظهر التحليل وجود ٨ معلمات جزيئية سالبة موجودة فى الأب الحساس ذات أحجام (٤٥٣ ، ٧٧٠) زوج قواعد للبادئ B17 و(٩٧٥ ، ١٤٣٣) زوج قواعد للبادئ B20 و ٢٥٢ زوج قواعد للبادئ F05 و(٧٦٨ ، ٢٤٠٨) زوج قواعد للبادئ F06 وأخيراً ٢٢٥ زوج قواعد للبادئ F08.