GENETIC ANALYSIS OF PEROXIDASE AND ESTERASE ISOZYMES IN CALLUS INDUCED FROM MATURE EMBRYOUS OF WHEAT (*Triticum aestivum* L.) GROWN UNDER DIFFERENT LEVELS OF SALINITY

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ABSTRACT

Fifteen wheat (*T. aestivum*. L.) populations represented by five parents and their ten F_1's generations were used to study the molecular polymorphism of peroxidase isozymes and their response to salinity. zymograms of peroxidaseand esterase molecular forms were obtained using the nondissociating P A G E technique.

Appearance, disappearance and intensity variation of isoperoxidases and isoesterases in embryo callus tissues after saline treatments suggest that either increasing and suppressing transcription or translation of these isozymes are observed after treatments by seawater. This suggests that salinity might stimulate the induction of many elicitors, thereby, affecting the regulation mechanism of the peroxidase and esterase isozymes loci, causing a transitory inhibition of some molecular forms.

The peroxidase electrophoresis system declared that the salt treatments had a decreasing effect for Giza 164 and Sakha 69 varieties, while the mutant lines I.A. 51 and I.A.76 were adopted with the salinity stress. The cross between Sakha 8 x I.A. 51 gave the greatest number and high intensity of bands, while Sakha 8 x Sakha 69 had the least number and lowest intensity of bands. The presence of peroxidase molecular forms at 3.2 and 3.5 cm in most crosses in spite of their absence in their parent under saline treatments, clearly suggest that these bands could be considered as salt shock protein bands which appeared after saline treatment, thereby the peroxidase isozyme pattern could be used as a good marker to distinguish the plant tolerant and/or resistance to salinity.

The zymograms of isoesterase showed that, the lines I.A.51 and I.A.76 were distinguished with stability against the environmental salinity, while the variety Sakha 69 had the lowest number of esterase bands with low intensity. Meanwhile, the cross I. A. 51 x I. A. 76 was the best hybrid, which had the greatest number of bands. Also the electrophoretic profile of esterase isozyme under non-saline conditions revealed that the general variation between the control and salt treatments represented in the four bands at positions 0.3, 0.5, 5.6 and 5.8 cm which were appeared only under all the salt treatments. These results clearly suggested the presence of an association between salt tolerance and the presence of these bands. In addition the esterase isozyme system was a reliable system for discriminating parents and their hybrids under saline condition.

Key words: Callus, embryo culture, esterase, genetic analysis, isozymes, peroxidase, salinity, wheat

INTRODUCTION

The electrophoretic techniques were found to be a useful tool for the detailed studies of seed proteins and isozymes. Electrophoretic banding patterns of isozymes have found to be useful for identification and

characterization of particular genotypes and also in establishing the predominance of the banding pattern of one or the other parent in the hybrids (Frei *et al*. 1986). This technique could contribute to detect not only the qualitative variabilities “presence / absence of bands” but also quantitative “variation in band intensities” among genotypes (Amot, 1992).

Here we are also interested in utilizing specific biochemical technique as polyacrylamide gel electrophoresis (PAGE) to examine wheat isozymes and their relationship with salt tolerance. This relationship could be used by the breeder in establishing strategies for selecting early generation materials in developmental programs of varieties.

The objective of this investigation is to develop biochemical genetic marker such as isozyme electrophoretic profiles of peroxidase and esterase to discriminate between these parental genotypes and their hybrids under normal and salinity conditions.

MATERIALS AND METHODS

This investigation was carried out at the Agricultural Botany Department, Faculty of Agricultural, Suez Canal University.

1. Materials:

The materials used in this investigation included two homozygous mutant lines of wheat (*T. aestivum* L.) produced from Giza 157 by EMS treatment since fifteen years ago and is characterized by high tolerance or resistance to salinity (Abd El-Raheem, 1990), as well as three different cultivar varities namely Sakha 8, Sakha 69 and Giza 164 which were characterized by tolerant, moderate tolerant and sensitive to salinity, respectively. The F_1 hybrids derived from diallel crosses were also used.

2. Methods :

Peroxidase and esterase isozyme systems were screened in callus tissue produced through embryo culture technique using four levels of sea salts (0.0, 7000, 9000 and 11000 ppm) in the media in all material genotypes at the Genetic Lab., Botany Department, Faculty of Agriculture, Suez Canal University.

A. Enzyme extraction:

Equal weight of fresh samples (0.5 gm) were taken and crushed directly in an ice-cold (0 – 4˚C) 1 M tris buffer, pH 7.8. The enzyme extraction buffer and procedures were applied according to Tanksley and Orton (1983).

B. Gel preparation, sample loading and electrophoresis:

A stock of 30% acrylamide, N’ N’ Bis methylene acrylamide stock (30% T and 2.67 % C) was used for preparing a 15% discontinuous, non-dissociating polyacrylamid molds. TEMED and ammonium persulfate were added to initiate polymerization of acrylamide in a tris-borate buffer pH 8.6. The gel mixture was loaded in a 20 x 20 cm vertical slab cell. A total of 40 µl

(25 µl of sample in crushing buffer and 15 µl of 10% sucrose in 0.2% bromophenol blue solution) was loaded in each sample slot. Electrophoresis was carried out using constant voltage of 250 DC volts. Eight hours were needed for each run.

C. Staining and data collection :

Peroxidase isozymes were stained according to Guikema and Sherman, 1980. The esterase isozyme bands were stained according to Kahler and Allard (1970) with some modification suggested by Tanksley and Rick (1980). The distance traveled by isozyme bands were measured in cm and recorded directly on graph paper as relative mobility values, Rm, then the gels were photographed.

RESULTS AND DISCUSSION

A. Peroxidase isozyme polymorphism:

The electrophoretic banding pattern of peroxidase for the five parents and their ten hybrids which analyzed from callus tissues of wheat embryous were represented by photographs of figures 1, 2 and 3 and zymograms of Figures 7, 8 and 9. The results indicated that peroxidase isozymes had five anodal bands. The zymograms demonstrated differences between the parental genotypes and their hybrids in number, positions and intensity of the bands either under normal or salinity conditions.

The zymograms of the variety Giza 164 (Fig. 7) showed that both control and salt treatments (7000, 9000 and 11000 ppm) had bands at positions 2.3, 2.6 and 2.9 cm while the band at position 1.9 cm appeared at control and disappeared at salinity treatments. The zymograms of the parent Sakha 8 (Fig. 7) showed that both control and salinity treatments were collaborated in the bands at the positions 2.3, 2.6, and 2.9 cm, while the bands at the positions 2.0 and 3.2 cm were found to be only under salt treatments. The zymograms of the variety Sakha 69 (Fig. 7) showed that the bands at the positions 1.0, 2.0, 2.3 and 2.6 cm were appeared at all treatments, while the band at 1.9 cm position was found to be presence only at 9000 and 11000 ppm salt treatments. The zymograms of both parental mutant lines (Fig. 7) showed the absence of the band at the position 1.9 cm in I.A. 51 mutant line under 7000 and 9000 ppm treatments, and the absence of bands at the positions 1.9 cm and 2.9 cm in I. A. 76 mutant line under 11000 ppm treatment. However, the bands at the positions 2.0, 2.3 and 2.6 cm appeared under saline and non-saline treatment in both mutant lines. These results clearly indicates some sort of transient decrease in the expression of most isoperoxidase loci shortly after saline treatments, suggesting that salinity cause the induction of different elicitors which play a direct or indirect role in either transcription activation or suppression of alleles controlling these molecular forms or, at least in decreasing their transcription level. Similar results were also observed by Teramoto *et al* (1996).

The electrophoretogram of peroxidas in the cross Giza 164 x Sakha 8 (Fig. 7) showed that the bands at the positions 3.2 cm appeared only at 7000 ppm treatment, the bands at the positions 2.3 and 2.6 appeared at all treatments, the bands at the position 1.0 and 2.0 cm appeared under 9000 and 11000 ppm, the band at 1.9 cm position appeared only under 11000 ppm treatments and the band at the position 2.9 appeared under control and 7000 ppm, while disappear under 9000 and 11000 ppm.

The zymograms of the hybrids Giza 164 x Sakha 69 and Giza 164 x I.A. 51 (Fig. 8) showed that both control and salt treatments were collaborated in the existence of the bands at the positions 2.3, 2.6 and 2.9 cm, the bands at position 3.2 cm appeared under 9000 ppm in both crosses and under 11000 ppm in the cross Giza 164 x I.A. 51, while the band at the position 3.5 appeared only under 9000 ppm salt treatment in both crosses. The band at position 1.0 cm appeared in the cross Giza 164 x I.A. 51 under control, 7000 and 11000 ppm. The zymogrames of cross Giza 164 x I.A. 76 (Fig. 8) showed that the bands at positions 2.6 cm and 2.9 cm appeared at all treatments, while the bands at positions 3.2 cm and 35 cm appeared under all treatments except under 7000 ppm salt treatment and the band at position 2.3 appeared under saline treatments only. The zymogram of the F_1 hybrid Sakha 8 x Sakha 69 (Fig. 8) showed that both control and salt treatments were collaborated in the bands at positions 2.0 cm and 2.3 cm, while the bands at positions 1.0 cm 3.2 cm and 3.5 cm appeared only under 7000 ppm salt treatment. On the other hand the band at position 2.6 appeared under control and 11000 ppm and the bands at positions 1.9 cm and 2.9 cm appeared only under control. The zymogram of the hybrid Sakha 8 x I.A. 51 (Fig. 8) showed that both control and salt treatments had the bands at positions 2.3 cm, 2.6 cm and 2.9 cm. However, the bands at positions 3.2 cm and 3.5 cm appeared only under saline treatments. The zymogram of the F_1 hybrid Sakha 8 x I.A. 76 showed that all treatments were collaborated in the presence of the bands at positions 2.0 cm, 2.3 cm and 2.6 cm, while the bands at position 1.0 cm and 2.9 cm appeared under control and disappeared under all saline treatments and the band at position 1.9 cm appeared under salinity conditions only. (Fig. 9) represent the zymograms of the crosses Sakha 69 x I.A. 51, Sakha 69 x I. A.76 and I.A. 51 x I.A. 76. The zymograms revealed that the three hybrids were collaborated in the existence of the bands at positions 2.3 cm and 2.6 cm. For the cross Sakha 69 x I.A. 51 the bands at position 1.2 cm and 1.4 cm appeared under control only, the band at position 2.0 cm appeared under all treatment except under 11000 ppm salt treatment, the bands at positions 2.9 cm, 3.2 cm and 3.5 cm appeared only under saline conditions. For the cross Sakha 69 x I.A. 76 the bands at positions 2.0 cm and 3.2 cm appeared only under salt treatments and for the cross I.A. 51 x I.A. 76 the zymogram showed that the band at position 1.4 cm appeared only at control, while the band at the position 3.2 cm appeared only under 9000 ppm salt treatment.

Generally, the salt treatment had a decreasing effect on the variety Giza 164 and Sakha 69, while the mutant lines I.A. 51 and I.A. 76 were adopted with saline stress. On the other hand, the mutant lines and the variety Sakha 8 had the greatest number and high intensity of bands under all

saline treatments compared with the other variety used. Moreover, the crosses between the mutant lines and Sakha 8 had the largest number of bands with higher intensity, while the other crosses gave least number of bands and lowest intensity. Similar results was obtained by Garcia *et al*. (1986) who found that the peroxidase enzyme activity were higher with NaCl treatments. Moreover, Li (1992) found that peroxidase activity increased in salt sensitive callus which were grown in a medium contained NaCl salt. Finally the results of peroxidase isozymes showed inconsistent banding patterns between control and salt treatments for all parents. While in some hybrids the bands at the positions 3.2 cm and 3.5 cm could be considered as salt shock protein bands after salt treatments. For this reason the peroxidase isozyme patterns could be used as a good marker to distinguish among hybrids but not among parents.

B. Esterase isozyme polymorphism:

For esterase polymorphism in relation to salinity, the zymograms of esterase isozymes under control and saline treatments in the parental and F_1 generations were presented by photographs Figures (4, 5 and 6) and zymograms in Figures 10, 11 and 12.

The differences between the parents and their F_1's , and between F_1's for number and type of bands were observed under control and salt treatments. However, the bands at positions 2.0, 2.2, 3.4, 4.7, 4.9 and 5.2 cm were appeared either under normal or saline treatments for the parents and their F_1 generations.

The zymogram of the variety Giza 164 (Fig. 10) showed that the non-saline treatment contained 13 bands at the positions 2.0, 2.2, 2.8, 3.0, 3.4, 3.6, 3.8, 4.5, 4.7, 4.9, 5.2, 5.4 and 5.6. the salt treatment 7000 ppm contained the same bands except the two bands at the positions 2.8 and 3.0 cm, while the salt treatment 9000 ppm contained the same bands which found under normal condition, meanwhile the salt treatment 11000 ppm contained 12 bands, the same bands which found under the treatment 7000 ppm except the band at the position 3.8 cm in addition to two bands at the positions 0.3 and 0.5 cm. The comparison between the banding pattern under control and highest salinity treatment 11000 pp, showed the absence of the isoesterase bands at the positions 2.8, 3.0 and 3.8 under 11000 salt treatment, while the bands at the positions 0.3 and 0.5 cm appeared only under this treatment.

The zymogram of the variety Sakha 8 (Fig. 10) showed that both control and salt treatments had eleven bands at positions 0.3, 0.5, 2.0, 2.2, 3.4, 3.6,. 4.5, 4.7, 4.9, 5.2, and 5.4 cm. However, the bands at positions 2.8 and 3.0 cm were observed under non-saline treatment and disappeared under saline treatments.

The zymogram of the variety Sakha 69 (Fig. 10) showed that the banding patterns of all treatments were collaborated in existence the bands at positions 0.3, 0.5, 2.0, 2.2, 3.4, 4.5, 4.7, 4.9, 5.2 and 5.4 cm. The bands at positions 2.8 and 3.0 cm appeared after saline effect, while the bands at positions 3.8 and 5.6 cm disappeared.

The zymogram of the mutant line I.A. 51 (Fig. 10) showed that the control and salt treatments had the same isoesterase bands at the positions

2.0, 2.2, 2.8, 3.0, 3.4, 3.6, 3.8, 4.5, 4.7, 4.9, 5.2, 5.4 and 5.6 cm. The variation between the control and salt treatment were fund to be at the positions 0.3 and 0.5 cm which appeared in all salt treatment, while disappeared in the control.

The esterase isozymes zymogram for mutant line I.A. 76 (Fig. 10) indicated that all salt treatments were similar in number and positions of bands (17 bands) 0.3, 0.5, 1.0, 1.2, 2.0, 2.2, 2.8, 3.0, 3.4, 3.6, 3.8, 4.5, 4.7, 4.9, 5.2, 5.4 and 5.6 cm while the control contained just 11 bands at the positions 2.0, 2.2, 3.4, 3.6, 3.8, 4.5, 4.7, 4.9, 5.2, 5.4, and 5.6. This indicates that the isoesterase variant at positions 0.3, 0.5, 1.0, 1.2, 2.8 and 3.0 cm appeared only under saline conditions.

Comparing between the five parents which were used in this investigation under non-saline condition showing that most parental genotypes had the same number of bands. After salt treatments observed that there was changeable in number and density of the bands, this could be suggest that the saline effect either suppress the esterase loci preventing the transcription of some loci causing the absence of these isoesterases as shown in (Fig. 10) or may produce some elicitors which in turn cause the inhibition of some isoesterases. In this concern, the tolerant parents I.A. 51 and I.A. 76 had the greatest different in number and intensity of their bands compared with the sensitive varieties Sakha 69 and Giza 164. On the other hand this mean that, salinity stress stimulated some genes involving resistance of salinity in lines I.A. 51 and I.A. 76.

The zymogram of F_1 Giza 164 x Sakha 8 (Fig. 10) showed that the control and all salt treatments were similar in the bands at positions 0.3, 0.5, 1.0, 1.2, 2.0, 2.2, 3.4, 3.6, 3.8, 4.5, 4.7, 4.9 and 5.2 cm while the bands at position 5.4 was found to be absent after saline treatments.

Esterase electrophoretogram of the cross Giza 164 x Sakha 69 is presented in (Fig. 11). It showed that the control contained 12 bands at positions 2.0, 2.2, 2.8, 3.0, 3.4, 3.6, 3.8, 4.5, 4.7, 4.9, 5.2 and 5.4 cm. All saline treatments had the same bands (16 bands) at positions 0.3, 0.5, 2.0, 2.2, 2.8, 3.0, 3.4, 3.6, 3.8, 4.5, 4.7, 4.9, 5.2, 5.4, 5.6 and 5.8. the general variation between the control and salt treatment in this cross represented in the four bands at positions 0.3, 0.5, 5.6, and 5.8 cm, which were appeared after exposure to saline effect.

The zymogram of esterase isozyme for F_1 Giza 164 x I.A. 51 is given in (Fig. 11) indicated that control and saline treatments were collaborated in the bands at positions 2.0, 2.2, 2.8, 3.0, 3.4, 3.6, 3.8, 4.5, 4.7, 4.9, 5.2 and 5.4 cm, while it was different in the bands at positions 0.3, 0.5, 1.0 and 1.2 cm which were observed after saline treatments. Such variation in the electrophoretic patterns of peroxidase and esterase isozymes studied can exist if it is assumed that the genes responsible for these metabolic phenomena are different in their action. A reasonable explanation is that this cross has the highest tolerant parent (I.A. 51) and most sensitive parent (Giza 164) to salinity.

Esterase electrophoretogram of the cross Giza 164 x I. A. 76 (Fig. 11) showed the presence of the isoesterase variant at the positions 5.6 and

5.8 cm under all saline treatments while the bands at positions 2.0, 2.2, 2.8, 3.4, 3.6, 3.8, 4.5, 4.7, and 4.8 were similar in all treatments.

The zymogram of the hybrid Sakha 8 x Sakha 69 (Fig. 11) showed the similarity between the three treatments in the presence of the bands at the positions 0.3, 0.5, 2.0, 2.2, 2.8, 3.0, 3.4, 3.6, 3.9, 4.5, 4.7, 4.9, 5.2, 5.4, 5.6 and 5.8. The control had just 12 bands and the bands at the positions 0.3, 0.5, 5.6 and 5.8 were appeared after all saline treatment and disappeared under control.

The zymogram of the hybrid Sakha 8 x I.A. 51 (Fig. 11) showed that the control and saline treatments had 14 bands at positions 0.3, 0.5, 2.0, 2.2, 2.8, 3.0, 3.4, 3.6, 3.9, 4.5, 4.7, 4.9, 5.2 and 5.4 cm. The bands at positions 5.6 and 5.8 cm appeared only under saline treatments.

The electrophoretogram of esterase isozymes in cross Sakha 8 x I.A. 76 (Fig. 11) indicated that both control and salt treatments contained stable number of bands (16 bands) at positions 0.3, 0.5, 1.0, 1.2, 2.0, 2.2, 2.8, 3.0, 3.4, 3.6, 3.8, 4.5, 4.7, 4.9, 5.2 and 5.4 cm.

The zymogram of esterase isozymes for cross Sakha 69 x I.A. 51 (Fig. 12) showed the similarity between non-saline and saline treatments for the bands at positions 2.0, 2.2, 3.4, 3.6, 3.8, 4.5, 4.7, 4.9, 5.2, 5.4 and 5.8 cm. Meanwhile, isoesterase molecular form at positions 0.3 and 0.5 cm were observed after all saline treatments.

The esterase zymogram of the cross Sakha 69 x I.A. 76 (Fig. 12) showed the appearance of the bands at positions 2.0, 2.2, 3.4, 3.6, 4.7, 4.9, 5.2 and 5.4 cm under control and saline treatments, while the bands at position 1.0, 1.2, and 3.8 cm were found to be absent after saline treatments. However, the band at position 3.9 cm was appeared after exposure to salinity. The appearance or disappearance of different isoesterase molecular forms, as a result of saline effect, clearly suggested that a group of elicitors might be induced thereby affect the regulation mechanisms of isoesterase loci during and after exposure to salinity and/or interfere many metabolic pathways causing a transitory inhibition of some molecular forms of esterase. Similar conclusion was also reported by LaRosa *et al*. (1992).

The zymograms of the cross I.A. 51 x I.A. 76 (Fig. 12) showed that the control and all salt treatment were similar in the number of bands (18 bands) and the positions which were 0.3, 0.5, 1.0, 1.2, 2.0, 2.2, 2.8, 3.0, 3.4, 3.6, 3.8, 4.5, 4.7, 4.9, 5.2, 5.4, 5.6, and 5.8 cm. This result strongly suggest that the similarity of isoesterase bands in number and position under saline and non-saline treatments in this cross may be due to the genetic homozygosity and development of their parent as well as the parental genotypes had most alleles responsible for saline resistance.

Summing up these results, it might be beneficial to declare that the isoesterase zymogrames either for the parental mutant lines I.A. 51 and I.A. 76 or each hybrid included were distinguished with stability against the environmental salinity stress, while the variety Sakha 69 had the lowest number of bands and low intensity. Meanwhile, the cross I.A. 51 x I.A. 76 was the best hybrid, which had the greatest number of bands followed by Sakha 8 x I.A. 51 and Giza 164 x I.A. 76.

These results confirmed the finding of Singh *et al* (1985a) who noted that the intensity of bands increased when cell were cultured with increasing levels of NaCl adaptation. On the other hand, some intensities were reduced with the same conditions of salinity. Also, the elecrophoretic banding patterns of esterase isozymes under non-saline and saline treatments revealed that the general variation between the control and salt treatments represented in the four bands at positions 0.3, 0.5, 5.6, and 5.8 cm which were appeared only under all the salt treatments. These results clearly suggested the presence of an association between salt tolerance and the presence of these bands. These results confirmed the finding Salam (1993) who noted that some bands could be considered as a salt shock protein band which appeared after salt treatments. Finally, the esterase isozymes system was a reliable system for discriminating parents and hybrids under saline conditions.

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التحليل الوراثى للمشابهات الأنزيمية للبيروكسيديز والأستيريز فى الكالوس الناتج من أجنة القمح الناضجة النامية تحت مستويات ملوحة مختلفة

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تم استخدام خمس عشرة تركيب وراثى من القمح (*T. aestivum* L.) ممثلة بخمس أباء وعشرة هجين للجيل الأول وذلك لدراسة التعداد المظهرى للمشابهات الانزيميه لإنزيمي البيروكسيديز والاستيريز واستجابتها للملوحة وقد استعمل التفريد الكهربى لجيل البولى اكريلاميد للبروتينات الطبيعية الغير منفصلة وذلك للحصول على نموذج الأشكال الجزيئية لإنزيمي البيروكسيديز ولاستريز.
إن ظهور وعدم ظهور الحزم الخاصة بالمشابهات الإنزيمية للبيروكسيد والاستريز وكذلك تغير كثافة الحزم فى نسيج الكالوس الناتج عن زراعة الأجنة تحت معاملات الملوحة إنما يعزى إلى الزيادة أو النقص فى مستويات النسخ أو يعزى إلى مستويات مختلفة من التخليق والهدم لهذه المشابهات الانزيميه وذلك يقترح أن الملوحه ربما يتسبب عنها إنتاج عديد من المحثات التى تؤثر على آلية التحكم فى مواقع المشابهات الانزيميه للبيروكسيديز والاستريز مسببه تثبيطا مؤقتا لبعض الأشكال الجزيئية منها .
وقد أظهر نظام التفريد الكهربى للبيروكسيديز ان معاملات الملوحه كان لها تأثير بالنقص بالنسبه للصنفين جيزة 164 وسخا 69 بينما ظهر أن السلالتين I.A. 51 و I.A.76 كانتا متوائمتين مع ظروف الملوحه .
أعطى الهجين I.A.51 x سخا 8 أكبر عدد وأكثر كثافة للحزم بينما أعطى الهجين سخا 69 x سخا 8 أقل عدد وأقل كثافه للحزم .
أوضحت النتائج وجود حزمتين عند 3.2 سم و3.5 سم فى معظم الهجن بالرغم من غيابها فى آبائها تحت معاملات الملوحه مما يقترح أن هاتين الحزمتين تعتبران كاستجابه لصدمة الملوحة . وعلى ذلك فان المشابهات الانزيميه للبيروكسيديز يمكن أن تستخدم كعلامة جزيئية على التحمل و أو المقاومه للملوحه
لقد أظهر نموذج التفريد الكهربى للاستيريز أن السلالتين I.A.51 و I.A.76 تتميزان بالثبات للضغوط البيئيه للملوحه بينما كانت الحزم الخاصه بالصنف سخا 69 ذات كثافة منخفضة . وقد كان الهجين I.A.76 x I.A. 51 أفضل الهجن حيث أعطى أكبر عدد من الحزم ، كما أظهر نموذج تفريد الاستريز تحت ظروف الملوحة وعدم الملوحة أن الفروق بين معاملات الملوحة وعدم الملوحه تتضح فى الحزم عند المواقع 0.3سم ، 0.5سم ،5.6 سم ، 5.8 سم والتى ظهرت فقط تحت معاملات الملوحة ، وذلك يقترح وجود علاقه بين تحمل الملوحة وظهور هذه الحزم . وعلى ذلك فانه يمكن استعمال نموذج التفريد الكهربى للمشابهات الانزيمية للاستريز لتمييز الآباء والهجن الناتجه عنها تحت ظروف الملوحة .