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Study the Genetic Mode of Action Responsible for Germination Seeds of Oats (*Avena sativa*) under Low and High Levels of Temperatures

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ABSTRACT

Oats (Scientific name: *Avena sativa*) is a herbaceous species from around grassy plant, and is a sort of grain, seeds are used in human and animal nutrition, especially poultry and horses. Straw is sometimes used as a platform for living animals. Here are many uses and rolling at the moment on the derivatives oats but not scientifically proven, but it has a good positives, such as the use of oats as an antidote to stress, insomnia, calming and mascot to sleep and tonic for the nerves. Indians also used to treat oats opium addition and tobacco products, so, this study used the genotypes; Gerald, Millennium, Pambo-37, Sileton-18, Hendon and GXY-2000 which were grown under greenhouse conditions under two level of temperatures (25°C and 37°C) at rate of three replicates for each degree of temperature using randomized complete block design in Giza city to know the effect of different levels of temperatures through studying SDS-protein electrophoresis(water soluble protein), antioxidant enzyme (peroxidase and polyphenol oxidase) isozymes in addition to randomly amplified polymorphic DNA (RAPD-PCR) markers namely; (SRH-13), (MOR-7), (PAL-15) and (PAL-18) primers to know also, differences and relationships between these cultivars of oats (*Avena sativa*). The results revealed that the degree 25°C was optimum and importance for the genetic mode of action responsible for germination seeds without effect on the characteristics of water soluble protein and the efficiency of the enzymes responsible for the germination test, while the germination in the level of 37°C adversely effect on biochemical traits. Finally, biochemical parameters as alterations in the leaves of oats during protein banding patterns and change in the expression level of antioxidant enzymes were differed in the different conditions of temperatures.

Key words: Oats (*Avena sativa),* SDS-protein electrophoresis-peroxidase and polyphenol oxidase isozymes RAPD.PCR analysis.

Introduction

The wild ancestor of *Avena sativa* and the closely related minor crop, *A. byzantina*, is the hexaploid wild oat *A. sterilis*. Genetic evidence shows the ancestral forms of *A. sterilis* grew in the Fertile Crescent of the Near East. Domesticated oats appear relatively late, and far from the Near East, in Bronze Age Europe. Oats, like rye, are usually considered a secondary crop, i.e., derived from a weed of the primary cereal domesticates wheat and barley. As these cereals spread westwards into cooler, wetter areas, this may have favored the oat weed component, leading to its eventual domestication. Oats have numerous uses in foods; most commonly, they are rolled or crushed into oatmeal, or ground into fine oat flour, Gorham and Chapman, (1992).Oatmeal is chiefly eaten as porridge, but may also be used in a variety of baked goods, such as oatcakes, oatmeal cookies, and oat bread. Oats are also an ingredient in many cold cereals, in particular muesli and granola. Historical attitudes towards oats have varied. Oat bread was first manufactured in Britain, where the first oat bread factory was established in 1899. In Scotland, they were, and still are, held in high esteem, as a mainstay of the national diet.In Scotland, a dish called cow pat was made by soaking the husks from oats for a week, so the fine, floury part of the meal remained as sediment to be strained off, boiled and eaten. Oats are also widely used there as a thickener in soups, as barley or rice might be used in other countries. Oats are also commonly used as feed for horses when extra carbohydrates, and the subsequent boost in energy, are required. The oat hull may be crushed ("rolled" or "crimped") for the horse to more easily digest the grain[1] or may be fed whole. They may be given alone or as part of a blended food pellet, Hoffenberg *et al.,*(2000) Cattle are also fed oats, either whole, or ground into a coarse flour using a roller mill, burr mill, or hammer mill. Winter oats may be grown as an off-season groundcover and ploughed under in the spring as a green fertilizer, or harvested in early summer. They also can be used for pasture; they can be grazed a while, then allowed to head out for grain production, or grazed continuously until other pastures are ready.Oat straw is prized by cattle and horse producers as bedding, due to its soft, relatively dust-free, and absorbent nature, Ruenala *et al.,* (1998). The straw can also be used for making

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corn dollies. Tied in a muslin bag, oat straw was used to soften bath water. Oats are also occasionally used in several different drinks. In Britain, they are sometimes used for brewing beer Partridge and Whitcut ,(1995). Oatmeal stout is one variety brewed using a percentage of oats for the wort. The more rarely used oat malt is produced by the Thomas Fawcett & Sons Maltings, and was used in the Maclay Oat Malt Stout before Maclays Brewery ceased independent brewing operations. A cold, sweet drink called *avena* made of ground oats and milk is a popular refreshment throughout Latin America. Oatmeal caudle, made of ale and oatmeal with spices, was a traditional British drink and a favourite of Oliver Cromwell.Oat extract can also be used to soothe skin conditions.Oat grass has been used traditionally for medicinal purposes, including to help balance the menstrual cycle, treat dysmenorrhoea, and for osteoporosis and urinary tract infection Janatuinens et al (1995) . In the United States, No.1 oats weigh 42 pounds per US bushel (541 kg/m^3); No.3 oats must weigh at least 38 lb/US bu (489 kg/m^3). If over 36 lb/US bu (463 kg/m^3), they are graded as No.4, and oats under 36 lb/US bu (463 kg/m^3) are graded as "light weight".In Canada, No.1 oats weigh 42.64 lb/US bu (549 kg/m^3); No.2 oats must weigh 40.18 lb/US bu (517 kg/m^3); No.3 oats must weigh at least 38.54 lb/US bu (496 kg/m^3) and if oats are lighter than 36.08 lb/US bu (464 kg/m^3) they do not make No.4 oats and have no grade.Note, however, that oats are bought and sold, and yields are figured, on the basis of a bushel equal to 32 pounds (14.5 kg or 412 kg/m^3) in the United States and a bushel equal to 34 pounds (15.4 kg or 438 kg/m^3) in Canada. Yields range from 60 to 80 US bushels per acre (5.2–7.0 m^3/ha) on marginal land, to 100 to 150 US bushels per acre (8.7–13.1 m^3/ha) on high-producing land. The average production is 100 bushels per acre, or 3.5 tonnes per hectare.Straw yields are variable, ranging from one to three tonnes per hectare, mainly due to available nutrients, and the variety used (some are short-strawed, meant specifically for straight combining) Comino et al (2011). The overall aim of this paper was to enhance the value of oats to cereal growers and meet the needs of end-users through the development of oat germplasm and genetic stocks leading to new varieties. Oats are a valuable crop due to their unique properties. As a result of discussions, we defined clear industry pull for genetic improvement for diverse industries including feed for poultry, oat milling, breakfast cereal manufacturing, functional foods and feeding to pigs and ruminants. Common requirements for all these varied end-users are improvements in agronomic characteristics, e.g. grain yield, increased resistance to lodging and greater pest and disease resistance.

Materials and Methods

Six cultivars of oats (*Avena sativa*) namely; Gerald, Millennium, Pambo-37, Sileton-18, Hendon and GXY-2000 grew in the greenhouse conditions under two level of temperature (25°C and 37°C) at a rate of three replicates for each level of heat using the design of randomized complete block to know the effect of different levels of temperature on the traits of protein and the efficiency of the enzymes responsible for a germination ideally through using SDS-water soluble protein, antioxidant enzyme peroxidase and polyphenol oxidase isozymes in addition to randomly amplified polymorphic DNA(RAPD)-markers namely; (SRH-13), (MOR-7), (PAL-15) and (PAL-18) primers to study differences and relationships between these genotypes of oats in the farm of Agriculture Research Center in Giza City and Department of Genetic and Cytology, Division of Genetic Engineering and Biotechnology, National Research Center, Dokki, Giza, Egypt during 2011 season.

Molecular markers Technique:

SDS-protein electrophoresis:

The leaves of Oats were used after 30 days from germination under the two levels of temperatures (25°C and 37°C) to study the protein banding patterns and protein fraction was performed according to the method of Laemmli (1970) and modified by studier (1973).

Isozymes electrophoresis:

Native-polyacrylamide gel electrophoresis (native-PAGA) was conducted according to Stegemann *et al.,* (1985) to identify isozyme variations between the two levels of temperatures responsible for the optimum germination on the leaves of the six genotypes of oats (*Avena sativa*) using two isozymes systems; peroxidase and polyphenol oxidase, respectively, (Brown, 1978).

RAPD-PCR-analysis:

In this experiment, we need to make RAPD-PCR technique to identify the bands which marker for each line of oats by using the four primers namely; (SRH-13), (MOR-7), (PAL-15) and (PAL-18), respectively.
DNA was extracted from the selected leaves of six lines of oats under the two level of heat (25°C and 37°C) according to the method of Williams *et al.,* (1990), Graham *et al*., (1997) and Sharma *et al.,* (2003).

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Cluster analysis:
Dendrogram was performed by Nei & Lis (1979) and Jaccard (1984).

Table 1. Gel electrophoretic buffers

TBE buffer	10X
Tris	10.80g
Boric acid	5.50g
EDTA	0.74g
H_2O(dd)	Up to 100m1
Loading buffer (Tris)	10.8g
Boric acid	5.5g
EDTA	0.74g
H_2O(dd)	Up to 100m1

Results and Discussion

Molecular markers:
SDS-protein electrophoresis:

The electrophoretic banding patterns of proteins extracted from the leaves of oats (*Avena sativa*) under both levels of temperatures were showed in Fig. 1 and Table 2). The bands number (4, 6, 7) with molecular weights of (70, 60, 35) KDa, respectively were appeared in all cultivars of oats under both levels of temperatures, which means that these bands were commonly bands in these lines. On the other hand, the bands number (1, 3, 5) with molecular weights of (112, 95, 65) KDa were observed in the genotypes number (P_1, P_2, P_3, P_4) for the First and second bands only and (P_1, P_4) for the third band under low level of temperature, respectively, while, all parents were showed for the bands number (1, 3) with molecular weights of (112, 95) KDa except the parents number (4, 5, 6) for the second band under high level of temperature, but, the band number 5 with molecular weight of (65)KDa was not appeared in all parents under the same condition, respectively. The appearance of the bands number (1, 3, 4, 6, 7) with molecular weight of (112, 95, 70, 60, 35) KDa in most of parents under high level of temperature maybe due to manufacture specific protein responsibility and powering for heat tolerance in oats. This increasing of density and intensity of the bands number (4, 6, 7) with molecular weight of (70, 60, 35) KDa in all parents under all conditions may be due to high ability of heat tolerance in these lines of oats during the stage of germination and this modification of gene expression is due to high conservative genes found in (*Avena sativa*). These genes might have a crucial role in the response to different stresses as well as the main role of systemic signals gene related by the tissue exposed to heat tolerance in oats. These results reported by El-Fadly *et al*. (2007), Al-Wahibi (2010) and El-Mouhamady *et al*. (2014).

Table 2. The protein banding patterns of SDS-PAGE of the genotypes of oats (*Avena sativa*) under low and high levels of temperature

Band No.	MW (KDA)	Low level of temperature						High level of temperature					
		P_1	P_2	P_3	P_4	P_5	P_6	P_1	P_2	P_3	P_4	P_5	P_6
1	112	+	+	+	+	-	-	+	-	+	+	+	+
2	110	-	-	-	-	-	-	-	-	-	-	-	-
3	95	+	+	+	+	-	-	+	+	+	-	-	-
4	70	+++	+++	+++	+++	+++	+++	++	++	++	++	++	++
5	65	+	-	-	+	-	-	-	-	-	-	-	-
6	60	+++	++	++	++	++	++	+	+	+	+	+	+
7	35	+	+	+	+	+	+	+	+	+	+	+	+
Total of Bands		6	5	5	6	3	3	5	4	5	4	4	4

(+) : very faint (++) : fain (+++) : very dark (-) : absence of bands
P1 : Gerald P2 : Millennium P3 : Pambo-3 P4 : Sileton-18
P5 : Hendo P6 : GXY-2000

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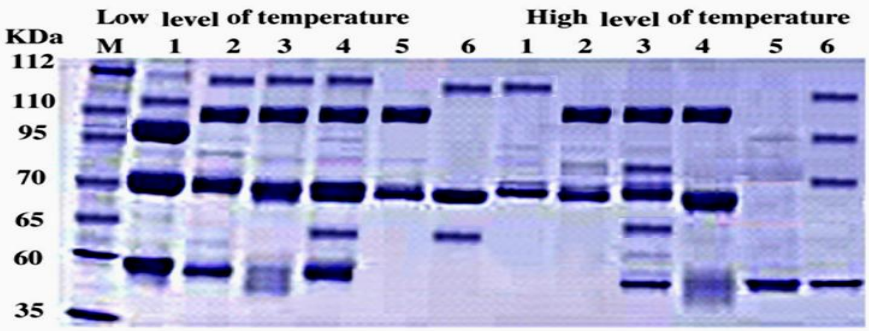
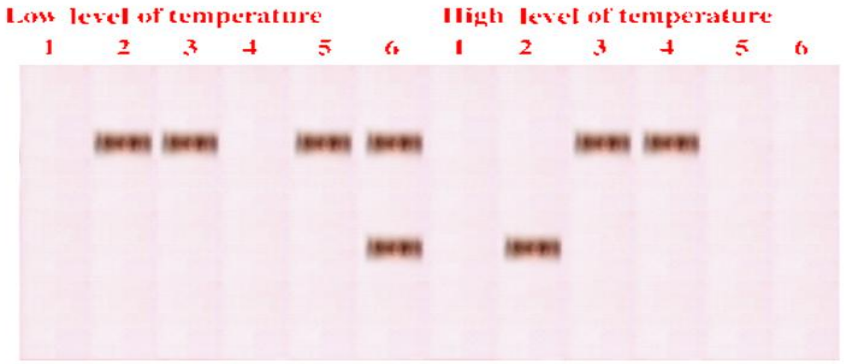


Fig(1):-SDS-PAGE of water soluble protein fraction of the genotypes of Oats (Avena Sativa) under low and High levels of temperature.

Isozymes electrophoresis:

Peroxidase Isozymes:

The electrophoretic patterns of peroxidase isozymes under the two levels of temperatures in oats were showed in table (4) and Fig. (2). A total number of four bands were exhibited; all bands were appeared in all parents of oats under low level of temperatures, while the bands number (1, 2) were observed in all parents under high level of temperatures only. The preview results indicated that the bands number (3, 4) disappeared under high level of temperatures as compared with the low level of temperatures. The reason for decreasing in peroxidase activity after exposure to high degree of temperatures in the bands number (3, 4) maybe due to damage of the protein which control for heat tolerance in oats. The results showed that all parents of oats were appeared in the bands number (1, 2) under high level of temperatures and were highly tolerance for heat tolerance when its were sufficient compatibility to express different reaction in high degree of temperature as compared with the low level of it, as well as antioxidant enzymes response to different ability for abiotic stresses and proved the favorable conditions to this protein in order to have their a little activity to neutralize the free radicals' which are produced under high level of temperature. High density of the bands (1, 2) in all parents of oats under the high level of temperature only maybe due to manufacture protein responsibility for tolerance to high level of temperature during the germination stage. Finally using of peroxidase isozyme as a marker for heat tolerance in oats and found that the profile of peroxidase enzyme was modified during stress of heat conditions, also a new subset of proteins induced by high level of temperature compared to normal temperature and this behavior may be due to its ability to heat tolerance or due to the effect of high temperature which may cause some shift in gene expression, El-Baz *et al*., (2003) and Roy and Mandal (2005).



Fig(2):-Electrophoretic patterns in Oats (Avena Sativa) for peroxidase isozymes under low and high levels of temperature.

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Polyphenol oxidase Isozymes:

Four bands appeared for polyphenol oxidase in oats under two levels of temperatures (low and high) Fig. 3 and Table (3), the band number one was appeared in all parents of oats under low and high levels of temperatures, which means that this band was common band for these cultivars under both conditions of temperatures. The appearance of these band under all conditions was different in densities and intensities especially under stress of temperature. While, the bands number (2, 3, 4) were appeared in the parents number; (1, 3) and (2, 3) for each band, respectively under low level of temperature only. It is noted that, failure to appear the bands number (2, 3, 4) in all parents of oats under stress of temperature maybe due to damage and influence the activity of enzymes and proteins responsible for the germination stage of oats, the variations occurred under stress of temperature compared with the control of temperature confirmed that its trigger the induction of compounds that regulate the induction or the activity of the tolerance of high temperature in oats. These results were in agreement with (El-Beltagi *et al.,* 2010) who found that the reason for decreasing in polyphenol oxidase activity after roasting maybe due to protein denaturation. Also (Goutom *et al.,* 1998) and (Montavon & Bortlik, 2004) reported that roasting treatments decline polyphenol oxidase activity in mushroom and coffee. The previous results are similar to the results of (Lee *et al., 2007*) who reported that antioxidant enzymes were up regulated under stress of drought in rice leaves, and also the enzymes related to metabolic pathway were differently accumulated for the ability of heat tolerance in oats (*Avena sativa).*
On the other hand, the highest activity in the two isozymes especially under stress of temperature in the band number (1) for all parents consistent with the results of (Nagesh and Devora J., 2008), who confirmed that quantitative and qualitative alteration in antioxidant enzyme system are often related to level of resistance to water stress, will quantitative changes in the enzyme level alterations observed in intensities and number of isozyme bands during applied stress and decrease of isozyme activity indicated gradual degradation of these enzymes on their structural modification under increasing heat tolerance in oats, whereas the banding pattern expressing differential intensity shows the varying status of enzyme affected by the stress of temperature. From the previous results it could be concluded that the percentages of the germination test at the level of 25°C were 96%,98%,99%,98%,97% and 98% ,while its were 55%,62%,54%,57%,55% and 50% at the level of 37 °C for the six cultivars of Oats,respectively.So the first level of temperature was optimum for germination of seeds in Oats because the activation of manufacture for protein to heat tolerance was very high and the activation of both enzymes was not damage on the contrary with the second level of temperature(37 °C) .

Table 3. Effects of the two levels of temperatures (low and high) on peroxidase and polyphenol oxidase isozymes in leaves of the six cultivars of oats (*Avena sativa*)

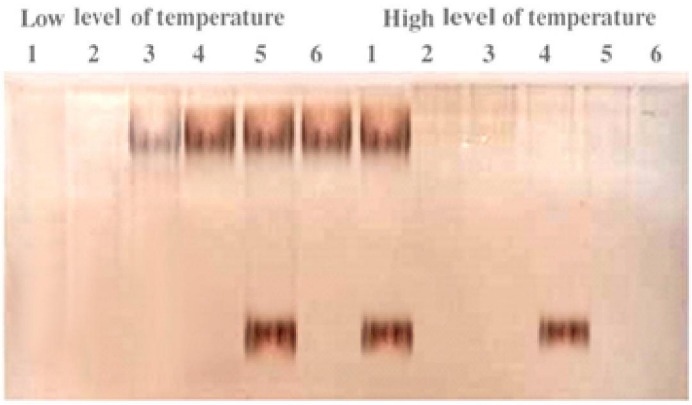
Band No.	Low level of temperature						High level of temperature					
	P_1	P_2	P_3	P_4	P_5	P_6	P_1	P_2	P_3	P_4	P_5	P_6
Peroxidase Isozymes												
1	++	++	++	+	+	+	+++	+++	+++	+++	+++	+++
2	+	+	+	+	+	+	+++	+++	+++	+++	+++	+++
3	+	+	+	+	+	+	-	-	-	-	-	-
4	+	+	+	+	+	+	-	-	-	-	-	-
Total	4	4	4	4	4	4	2	2	2	2	2	2
Polyphenol oxidase isozymes												
1	+	+	+	+	+	+	++	++	++	++	++	++
2	+++	-	+++	-	-	-	-	-	-	-	-	-
3	++	-	++	-	-	-	-	-	-	-	-	-
4	-	+++	+++	-	++	-	-	-	-	-	-	-
Total	3	2	4	1	2	1	1	1	1	1	1	1
Total	7	6	8	5	6	5	3	3	3	3	3	3

(+) : very faint (++) : fain (+++) : very dark (-) : absence of bands
P1 : Gerald P2 : Millennium P3 : Pambo-3 P4 : Sileton-18
P5 : Hendo P6 : GXY-2000

RAPD-PCR analysis:

The densitometric analysis of RAPD-PCR products in the six genotypes of oats using (SRH-13) and (MOR-7) primers are shown in table (4) and figures (4, 5), respectively.
The bands number (2, 3, 4, 5, 6 and 10) with molecular weights (3700, 1700, 1000, 800, 750, 120) bp were appeared in all cultivars of oats using (SRH-13) primer in Fig. (4) and were not appeared in the bands number (1, 7, 8, 9) with molecular weight of (4550, 500, 400, 200) bp, respectively, which indicated that these bands were primer and common bands for these lines of **o**ats, while, the bands number (6, 7, 8) with molecular weight of (450, 250, 200) bp were observed in all lines of oats in addition to the parents number (3, 4) were appeared by the band number (5) with molecular weight of 500bp only using (MOR-7) primer in Fig (5) in table (4), respectively, which means that these bands were commonly bands and marker in these cultivars of oats. On the other hand, the results in table (5) and Fig. (6) using (PAL-15) primer revealed that, the bands number (1, 2)

with molecular weight of (1450 & 1275)bp, respectively, were appeared in all lines of oats which means that these two bands were common bands and marker for these genotypes of oats, while, the bands number (5, 6, 7) with molecular weights of (750, 350, 250)bp were observed in the parents number (P3, P4, P5, P6) only, while, the bands number (4, 8, 9) with molecular weight of (900, 175, 100) bp were appeared in the cultivars number (P1, P2), (P1) and (P4) only, respectively, which means that these bands were commonly bands and marker for these genotypes of oats.



Fig(3):-Electrophoretic patterns in Oats (Avena Sativa) for polyphenol oxidase isozymes under low and high levels of temperature.

The bands number (1, 2, 5, 6, 7, 8, 9, 11, 13) with molecular weight of (1750, 1600, 1200, 1000, 800, 650, 550, 300, 50) bp were showed and appeared in the six parents of oats using (PAL-18) primer in table (5) and Fig. (7) respectively, which means that these bands were common bands and marker for these cultivars of oats, while, the bands number (3, 4, 10, 12) with molecular weight of (1550, 1400, 400, 150) bp were not appeared in all parents of oats.

Similar results are in agreement with those reported by Cheria & Fereira, (2010) who noted that heat-shock in the granule proteins of cupinesalbus was high significant using these primers to know common bands which were marker for heat tolerance under stress. The information gathered here would be helpful in genome mapping studies and for the development of oats cultivars with wider and diverse genetic background to obtain improved crop productivity during the optimum degree of germination. The data obtained in this experiment confirmed the efficiency of the RAPD technique for determination and estimation of genetic distance and relatedness among different plant genotypes. The RAPD analysis has been found to a valuable DNA marker system to evaluate genetic diversity. The information about genetic similarity will be helpful to avoid any chance of elite germplasm becoming genetically uniform, because of the simple experimental procedures, the requirement of minimal amount of plant tissue and the possibility of automation.

The results in Table (6) revealed that, four primers RAPD-PCR were used to identify six cultivars of oats and the total number of bands amplified per the four primers were 41band varied between (SRH-13) and (PAL-18) primers whereas, its were 13 and 18 bands, respectively, in addition to 27 band (Monomorphic) and 14 band were (polymorphic) resulting in a polymorphism of 55.5%. the size of bands varied between 4550bp and 50bp. The extent of polymorphism per primer ranged from 11% (PAL-15) primer to 55.5% (MOR-7) primer, on the other hand, both primers (SRH-13) and (MOR-7) scored zero unique marker sizes 4550 and 150bp, while, the primers (PAL-15) and (PAL-18) scored one unique marker with molecular 1450 and 50bp, respectively. RAPD-PCR analysis should be very useful in breeding for rapid and early identification of most diverse individuals in large seedling populations, allowing the detection of true to type genotypes for the improvement of our crop breeding programs. Keeping in view the useful information about the close genetic relationship, it is suggested that mission oriented breeding programs with the help of DNA fingerprinting technology will be helpful to produce distinct cultivars/genotypes with diverse genetic background and improved productivity.

Table 4. The densitometric analysis of RAPD-PCR products of the six cultivars of oats (*Avena sativa*) against (SRH-13) and (MOR-7) primers

Primer name	Base bairs	P_1	P_2	P_3	P_4	P_5	P_6
SRH-13	4550	-	-	-	-	-	-
	3700	+	+	+	+	+	+
	1700	+	+	+	+	+	+
	1000	+	+	+	+	+	+
	800	+	+	+	+	+	+
	750	+	+	+	+	+	+
	500	-	-	-	-	-	-
	400	-	-	-	-	-	-
	200	-	-	-	-	-	-
	120	+	+	+	+	+	+
	Total band	6	6	6	6	6	6
Primer name	Base bairs	P_1	P_2	P_3	P_4	P_5	P_6
MOR-7	1250	-	-	-	-	-	-
	1000	-	-	-	-	-	-
	850	-	-	-	-	-	-
	600	-	-	-	-	-	-
	500	-	-	+	+	-	-
	450	+	+	+	+	+	+
	250	+	+	+	+	+	+
	200	+	+	+	+	+	+
	150	-	-	-	-	-	-
	Total band	3	3	4	4	3	3

P1 : Gerald P2 : Millennium P3 : Pambo-3 P4 : Sileton-18
P5 : Hendo P6 : GXY-2000

Table 5. The densitometric analysis of RAPD-PCR products for the cultivars of oats (*Avena sativa*) against (PAL-15) and (PAL-18) primers

Primer name	Base bairs	P_1	P_2	P_3	P_4	P_5	P_6
PAL-15	1450	+	+	+	+	+	+
	1275	+	+	+	+	+	+
	1100	-	-	-	-	-	-
	900	+	+	-	-	-	-
	750	-	-	+	+	+	+
	350	-	-	+	+	+	+
	250	-	-	+	+	+	+
	175	+	-	-	-	-	-
	100	-	-	-	+	-	-
	Total band	4	3	5	6	5	5
Primer name	Base bairs	P_1	P_2	P_3	P_4	P_5	P_6
PAL-18	1750	+	+	+	+	+	+
	1600	+	+	+	+	+	+
	1550	-	-	-	-	-	-
	1400	-	-	-	-	-	-
	1200	+	+	+	+	+	+
	1000	+	+	+	+	+	+
	800	+	+	+	+	+	+
	650	+	+	+	+	+	+
	550	+	+	+	+	+	+
	400	-	-	-	-	-	-
	300	+	+	+	+	+	+
	150	-	-	-	-	-	-
	50	+	+	+	+	+	+
	Total band	9	9	9	9	9	9

P1 : Gerald P2 : Millennium P3 : Pambo-3 P4 : Sileton-18
P5 : Hendo P6 : GXY-2000

Genetic similarity matrix:

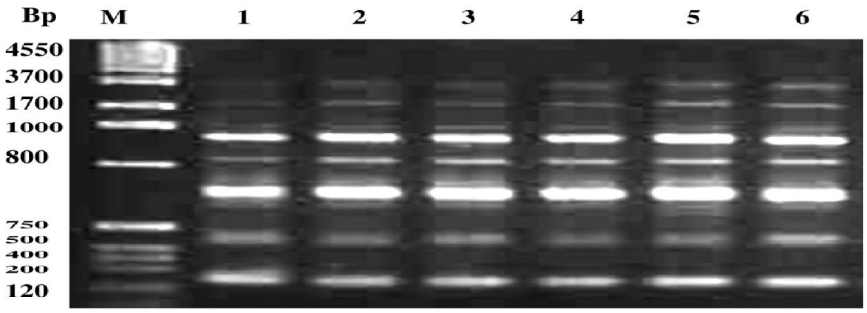
The dendrogram resulted from the combination between the banding patterns of protein SDS- PAGE and RAPD-PCR (Table 7 and Fig. 8), revealed that, six oats genotypes can be clustered in three distinct groups. Group A (similarity ranged from "0.74 to 0.93") contains on genotypes (Pampo-37) and (GXY-2000), Group B (Similarity ranged from "0.84 to 0.93") comprised of genotypes (Gerald, Sileton-18 and Hendon), while Group

C included on (Millennium) only and (Similarity ranged from "0.76 to 0.91), respectively. Molecular markers using RAPD-PCR showed better resemblance compared to biochemical markers using SDS-PAGE and isozymes. Thus, their disadvantages include a low level of polymorphism to have few alleles per locus, especially when the genetic base in narrow. In addition to, proteins can be affected qualitatively and quantitatively in their expression level by environmental factors and tissue type. On the contrary, molecular markers are not environmentally influenced, which means that the same banding profiles can be expected at all times for the same genotype (Kumar *et al.,* 2009). They indicated that RAPD-PCR technique can be used as a tool for determining the extent of genetic diversity among the six genotypes of oats and the importance of high level of genetic variability in the gene pool to face stress of temperature.

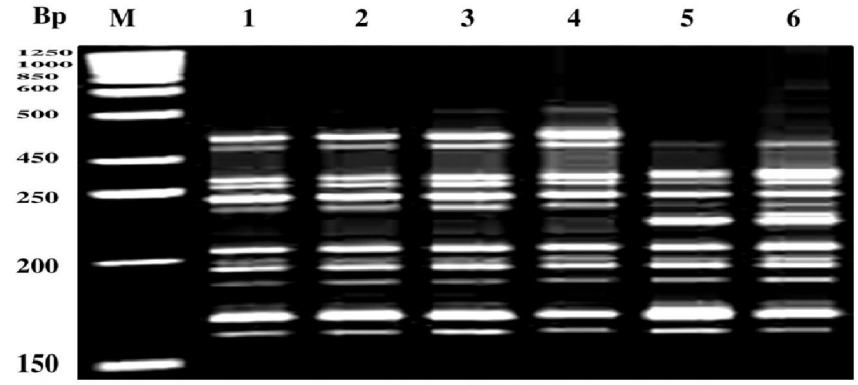
In the present study, the optimum temperature requirements for the germination stage of oats was a constant temperature of 25ºC, so, this has been clarified that the germination at 25ºC are ideal for germination process without effect on the characteristics of the protein and the efficiency of the enzymes responsible for a germination stage, while, the germination at the temperature of 37ºC adversely effect on biochemical traits responsible for it.

Table 6. Polymorphism of the RAPD-PCR primers among six oats genotypes.

Primer code No.	Sequence $(\grave{5} \rightarrow \grave{3})$	Size range of the Scorable Bands (bp)	Total bands	No. of monomorphic bands	No. of polymorphic bands	Unique bands	% polymorphism
SRH-13	ACCGTTATCG	4550-120	10	6	4	0	40
MOR-7	CTGGGTTCCA	1250-150	9	4	5	0	55.5
PAL-15	AGTCCGCAAC	150-100	9	8	1	1	11
PAL-18	GAGCCAATTCA	1750-50	13	9	4	1	31
Total			41	27	14	2	



Fig(4):-The densitometric analysis of RABD –PCR for the genotypes of Oats (Avena Sativa) using (SRH- 13) primer .



Fig(5):-The densitometric analysis of RABD –PCR for the genotypes of Oats (Avena Sativa) using (MOR- 7) primer .

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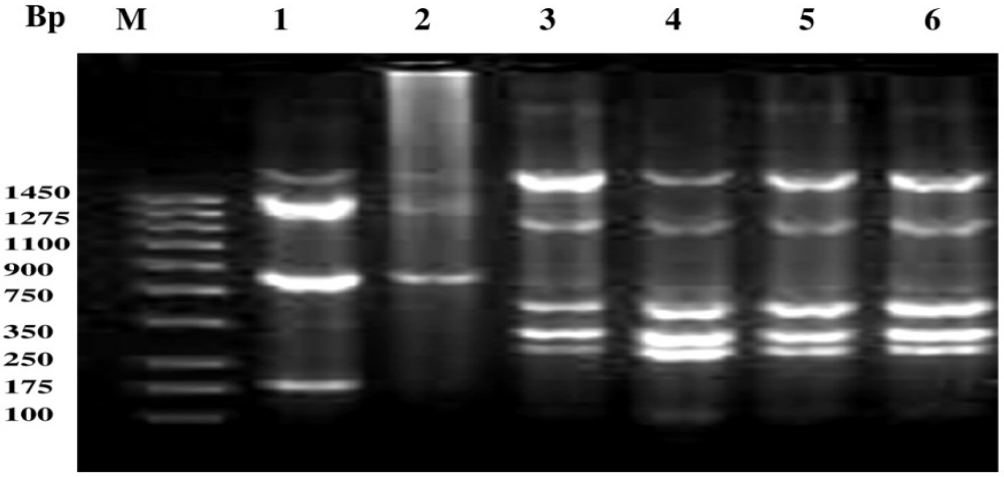
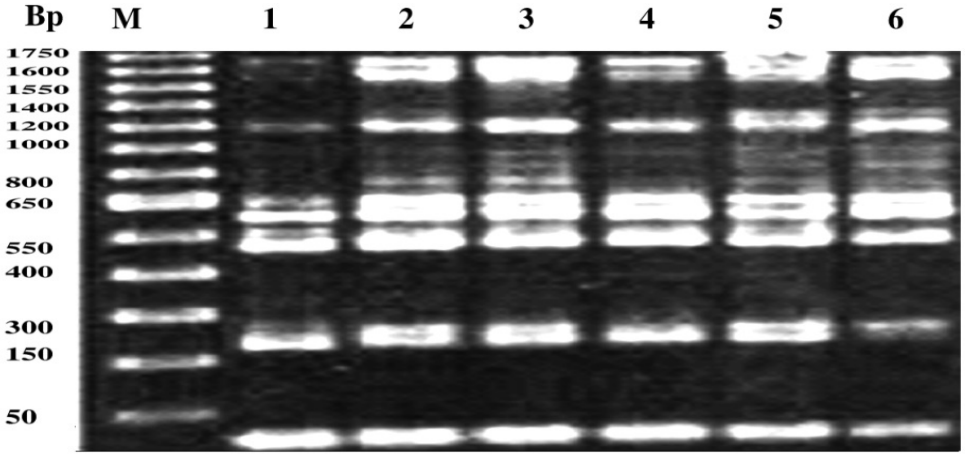


Fig(6):-The densitometric analysis of RABD –PCR for the genotypes of Oats (Avena Sativa) using (PAL-15) primer .



Fig(7):-The densitometric analysis of RAPD –PCR for the genotypes of Oats (Avena Sativa) using (PAL-18) primer .

Table 7. Similarity indices among six oats genotypes as estimated using SDS-PAGE and RAPD-PCR markers.

No.	P_1	P_2	P_3	P_4	P_5	P_6
P_1	1.0					
P_2	0.91	1.0				
P_3	0.86	0.89	1.0			
P_4	0.74	0.73	0.93	1.0		
P_5	0.69	0.91	0.78	0.75	1.0	
P_6	0.84	0.77	0.88	0.96	0.93	1.0

P1 : Gerald P2 : Millennium P3 : Pambo-3 P4 : Sileton-18
P5 : Hendo P6 : GXY-2000

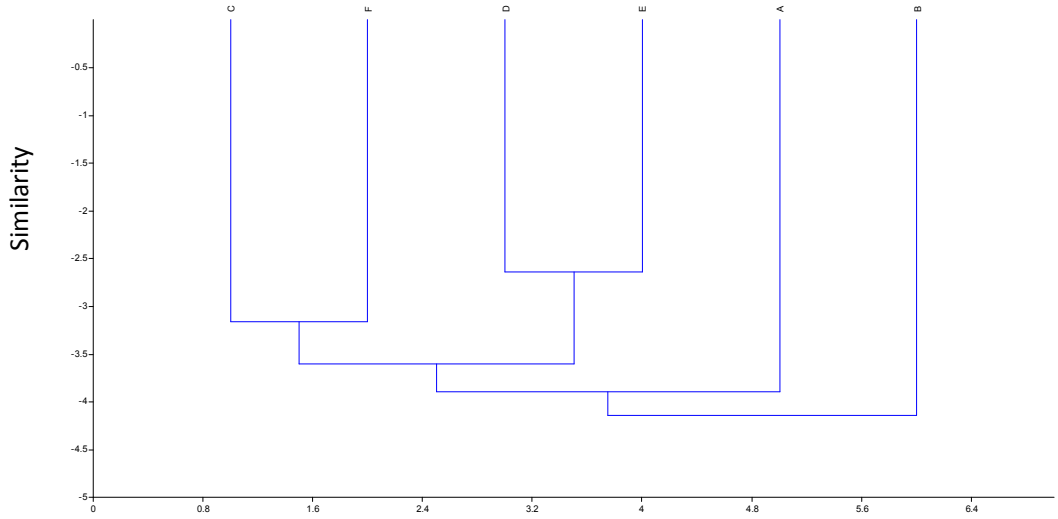


Fig. 8. Dendrogram of the six genotypes of Oats (*Avena Sativa*) namely,A (Gerald), B(Millennium), C(Pampo-37), D(Sileton-18), E(Hendon) and F(Gxy-2000), respectively, showing genetic distances and relationships among these genotypes based on SDS-PAGE and RAPD-PCR combination by UPGMA algorithm using Jaccard's similarity coefficient

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