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Performance and Determinate the Genetic Diversity of Different CowpeaProgenies Under Water Stress By Using SSR Markers.

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Abstract: *A field experiment was conducted at research station of College of Agriculture University of Baghdad in Abu-Griab. The aim was to study early generation of cowpea under water stress regime. The purpose was to identify which genotypes have high yield under water deficit. The experiment consisted of two levels of water deficit (50 and 75%) and three progenies selected and original cultivars. Randomized complete block design (RCBD) within split plots arrangement with three replicates in two seasons of 2014were used. The results revealed that progeny3 gave highest mean in chlorophyll index and78.8 SPAD in spring and autumn season respectively. In addition to, theprogeny3 had a short period reproductive phase. Progeny3 gave highest mean seed yield per hectare and water use efficiency were 0.222 and 0.193,0.702 and 0.585 in spring and autumn seasons, respectively. Progeny 3 gave less days to 50% flower were 56.67 and 41.73 in spring and autumn season, respectively. It can be concluded the significant dependence on certain traits in identifying the progenies that tolerated to the drought. Therefore,progeny3 was tolerant of drought because of superiority in relative water content. It is important to use progeny 3 in genotypic and environmental interactions for many years and locations to determine its stability. It can be rationed much quantity of water by following irrigation at 75%. Water deficit which reduced number of irrigation to nine in autumn season, leading to increase of water use efficiently to 0.585 in the autumn season under 75 % from soil of moisture.*

Simple sequence repeat (SSR) showed high levels of polymorphism between of between germplasms of cowpea, where the percentage of polymorphism ranged from 93% to 42%. The highest number of polymorphic bands (9) gave with primer SSR-6243. while the primer SSR-6211gave the lowest number of polymorphic bands (4). While the highest genetic distance between the progeny3 and origin cultivar about (5.56). The cluster analysis grouped the four germplasms of cowpea into two groups relied on the genetic and morphological traits. we concluded the PCR-SSR are helpful in identifying the genetic distance between progenies of cowpea and leads to select the progenies that may be used in production commercial cultivars and tolerate to biotic and abiotic stress.

Keywords:*PCR-SSR* **,***Cowpea progenies , Drought .*

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I. Introduction

Cowpea is one of the legumes crops includes 74 species of which three are commercially cultivated. unguiculate, sequedails, and cylindrica (Sardana et al, 1998). The cultivated area in the world is estimated about 12.5 million hectares. It's cultivated in the west and central Africa, which produces about 64% of the world's total production. The main producing countries are Nigeria and Senegal (Perino et al,1993). Cowpea like all field crops is exposed to abiotic stresses such as drought. Drought is a natural climate phenomenon that occurs frequently in the most parts of the world and can be agriculturally defined as the water stress in the soil to extent that plant cannot compensatethe evapotranspiration requirements (El-Shaieny et al, 2015). The problem of water supply shortages is a global problem,that does not concern a particular country, due to the multifaceted demand of water usage, massive population growth, and urban expansion. Therefore, plant breeders have to develop plants can grow and produce under limited water conditions. Plant breeders are trying currently to produce varieties of crops with the acceptable yield under drought conditions, thus saving a large amount of water and increasing the cultivated areas. The water stress can be divided into three levels according to the decrease of water potential values of plant tissue, which includes a mild stress: the water potential of cell is reduced by very few units measured by bar or by a decrease of 8-19 ° from dehydration under saturation, while moderate stress is represented the water potential of cell drops to less than 12-15 bar or (0-12%) decrease in water under saturation (Lin et al,1986).The sever water stress is referred to the water potential of cell drop to more than 15 bars and leads to a significant shortage of water saturation (Abed, 2014). The method of selection is effective in improving new genotypes based on the principle of gain from selection (AbodouRazakou et al,2013). Drought causes the low growth of roots, closure of stomata and reducing water loss through transpiration (Anyia and Herzog, 2004). Water variability is still one of the most important abiotic stress thataffectsgrowth and productivity of cowpea. However, varieties of cowpea are tolerant to drought as well as they have the ability to tolerate a wide range of soil acidity compared to other legumes (AbodouRazakou et al,2013; -Ahmed and Suliman, 2010). Many methods exist to the assessment of the genetic diversity in the cowpea progenies. Since the morphological traits are influenced by environmental conditions, they don't reliableto characterize the divergent in the crops. In this way, the evaluation based the morphological traits is veryexpensive and consuming more time, as well as the data are widely to select the genetically diverted in cowpea progenies. However, the genetic diversity between of the varieties are usually measured by the number of markers gene in the whole genome (Ogunkanni et al, 2008). DNA based markers to provide the useful information on the the genetic diversity to the cowpea varieties and the relationships between of the cowpea progenies developed from the the local of population or from the improved of cultivars. there are known to be tolerate to dry condition environmental conditions (Ogunkanni et al, 2008). SSR markers are highly informative and easy identified with the use of PCR- technique, as well as highly polymorphic, multi allelic and Randomly distributed throughout to the whole genome of plant, they are widely used to estimating the genetic diversity of the cowpea (Badiane et al,2012). This study was aimed (1) To evaluate the genetic diversity and polymorphisms to developing inbreds and commercial hybrids of cowpea. (2) To performance of early generations of self – pollination progenies and selection under water stress condition depend on the morphological traits.

II. Material and Methods

Field experiment:

A field experiment was carried out during spring and autumn of 2014 at research station of Agriculture College -University of Baghdad in order to evaluate the performance of selected generations of cowpea to tolerate dry conditions. The split plot arrangement was used inrandomized complete block design (RCBD) with three replications, the main plots were allocated from irrigation treatments (50% and 75% depletion) and subplots were allocated for (progeny1, progeny2, and progeny3) and origin cultivar (Bayader). The study farm was well prepared and divided according to the used experimental design. The experimental unit (3×3 m) consisted of four inner farrows; the seeds were planted at plant density of 25×25 cm inan average of 2 or3 seeds per hole (100 kg ha^{-1}). Soil samples were randomly taken and analyzed to determine some chemical and physical properties (Table 1). Nitrogen fertilizer as urea (46%N) wasadded in three partitions. The first portion was added during soil preparation, while the second part was added atelongation stage and the third part was added at flowering stage. Soil samples were also taken and placed in Petri dishes and weighed, then dried for 20 min in an oven, weighted again and finally, the moisture content was calculated according to method proposed by Zain(2002)

$$QV = QW \times ðb$$

Where

QV: Moisture content based on size

Qw: Moisture content based on weight

ðb:soil density $(m^3)^{-1}$

The irrigation was carried out with flexible plastic pipes and equivalent amounts of irrigation water were supplied to the field to ensure emergence of seedlings,according toKohnke equation (1968)

W= The amount of water to be added during the irrigation (m)

a= irrigation area

As = Soil density $(m^3)^{-1}$. megagram

%PWFC=percentage of soil moisture based on weight at field capacity before irrigation %PWW=percentage of soil moisture before irrigation

D=depth of total root (cm)

Field traits

1- Chlorophyll content index (SPAD)

The chlorophyll content of the leaves at physiological maturity stage was measured by chlorophyll meter $SPAD_{502}$ by taking three reading of three leaves from each branch, and average readings were calculated for five plants randomly.

2- The flowering of 50% from plants.

3- (POD) estimation in leaves according toAlsufi (2001)

4- Proline concentration estimation according to Bates et al. (1973)

lab experiment:

All material including 4 germplasms collected from cowpea progenies were used in this study. Seeds of these germplasms were grown in greenhouse and DNA was extracted from each germplasm according to Sambrook et al (1989). DNA concentration was 180-220 ng μL^{-1}. The markers was provided by Promega company to the Biotechnology lab in university of Al-Nahreen .(Table 1) and distributed widely of cowpea genome (1-10 chromosome).The reaction of PCR consisted of 2 µL of DNA solution ,1.2 ASB Buffer, 1.1 µL of 25 mM of MgCl2, 0.5 dNTP,1.0 µL from forward primer and 1.0 µL reverse primer, 0.2 Taq enzyme and 5.5 µL of double distilled water .The amplification reaction were carried out in a SSR as follows:

The reaction mixture was denatured at 95 ◦C for min initially, then subjected to 40 cycles 96 ◦C (1 min) ,50 ◦C (1min) and 72 ◦C (1 min), and final extension at 72 C for 5 min, prior to cooling at 5 ◦C. The PCR products was separated by electrophoresis 40 agrolmidgel With TBE 10 mL, APS with 1400 µl and EMET 40 µl. The amplified bands were recorded by "quantity one" system, then binary coded were (1) represented the presence band and (0) represented with not band in each germplasm. The polymorphism of each SSRproductwasdeterminate and described the genetic diversity between the germplasms by using Mega software(Cock et al ,2004)

Table1. Shown the numbers of SSR primers and their sequences that using to estimation genetic diversity.

Primers .codes	Sequence **(5'~3')**	Sequence **(3'~5')**	S. Complementary	length
SSR-6206	AGGCATGCATTCATCTTTCC	GCAGTCATAACCCCAAAACAA	0.00	20
SSR-6211	TGTCCTCAATTTCAATAACAAGTTT	AACAGTTGGTCGGATACGAAA	0.00	20
SSR-6243	GTAGGGAGTTGGCCACGATA	CAACCGATGTAAAAAGTGGACA	0.00	20
SSR-6257	TGCTTTTGTAAAAGGGTGGAA	ACTTGGACGGAACAGCAGAT	0.00	20
SSR-6281	GCATCAATTTGAGCGAGGAT	GAGTGACATTTCCGCGTCTT	0.00	20
SSR-6291	TCATGAGTTTCCACACACCAA	CCTTCGTATGTATATGTGGCTACTG		
Total				

III. Result and Discussion

Chlorophyll Content Index (CCI):

The Chlorophyll Content Index is a constant to determine plant response to the effectiveness in greenleaves; this, in turn, leads to delay of plant senescence, the elongate photosynthesis duration and movement ofmetabolitefrom source to sink (Tyagi et al, 2000). The results presented in figure1and 2. showedsignificant differences between the progenies and the origin cultivar. The progeny3 gave the highest mean of 76.7 SPAD, while the progeny1 gave the lowest mean of 70.94 SPAD. TheCCI behaved unstablyin generations compared to origin cultivar as it increasedby 3.8% on the origin cultivar under 50% depletion of soil moisture. In the autumn season, the same progeny3 was superior in comparison with the rest of genotypes and gave the highest average of 78.8 SPAD by 3.2% increasingpercentage. The higher CCI is due to the self-pollination of the selected plants, which led to an increase in gene frequency of stay - green genes (Thomas and Smart, 1993)

The depletion treatments weresignificantly varied in the Spring Season; the CCI was 75.9 SPAD when the water was depleted inan average of 50%. When the moisture was depleted by 75% the CCI was 71.02 SPAD, the stay- green increased with the increase of water quantity to the allowed limit, and thus reduce rolling of leaves and increase of the cell division and leave expansion (Johari-Pirevatlou et al, 2010). The progeny1 under 75% depletion of soil moisture gave an average of 73.0 SPAD. The decrease of the quantity of water irrigation can lead to a decrease of cell division and photosynthesis due to low CO_2 concentrations exchange in leaf tissues, which led to decreaseof CCI in leaves (Cardona et al, 2013)

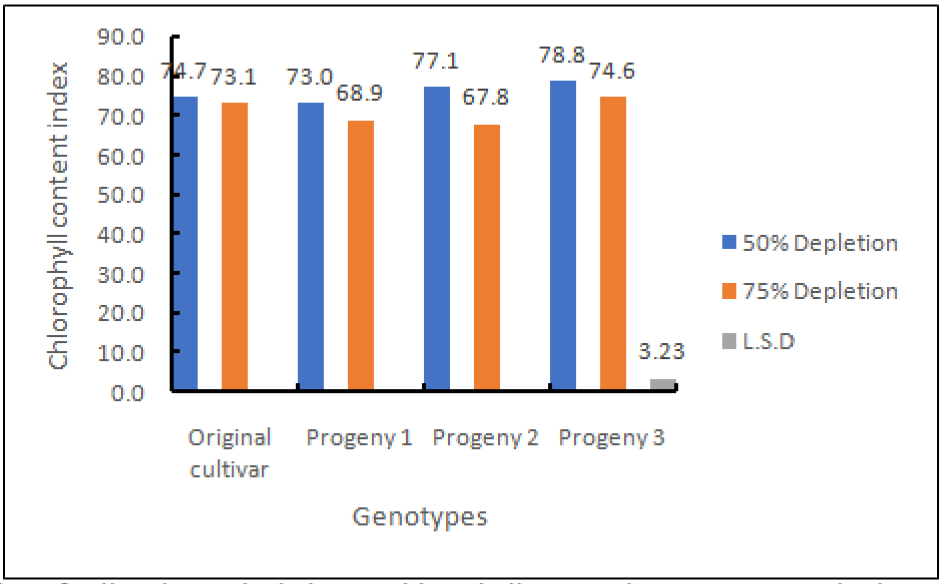

Fig 1. Effect of soil moisture depletion on chlorophyll content in cowpea progenies in spring 2014.

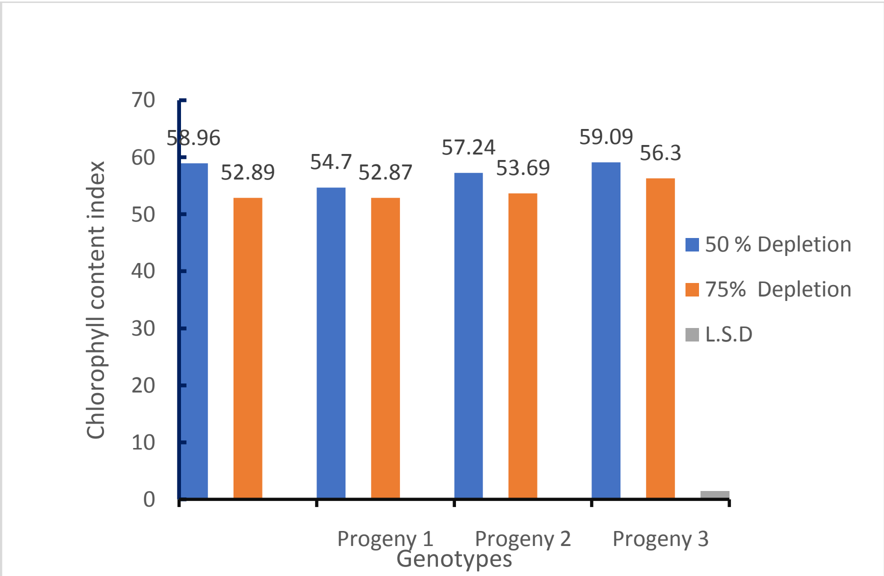

Fig 2. Effect of soil moisture depletion on chlorophyll content in cowpea progenies in autumn 2014.

Number of days to 50% flowering:

The flowering mechanism is a complex genetic trait in plants and controlledthe total plant yield. The results (Figure2) showed that there were significant differences between the progenies of cowpea with origin cultivar. The selected progenies were superior to theorigin cultivar by giving the lowest number of days until 50% flowering. The number of flowering days was slightly stable among generation compared to the origin, while, progeny2 decreased by 4.05% in early flowering compared to the origin cultivar. In Autumn Season, the same progeny2 has exceeded the rest of progenies, and giving the lowest average of 42.73 days and two days from the origin cultivar. The low number of days from planting to 50% flowering is associated with an increased gene frequency of pairs of genes, such as TFLI gene (terminal flower), which determines flowering time (El-Shaieny et al ,2015**)**

The depletion percentages significantly affected the flowering time, 75% depletion of soil moisture gave57.7 days compared to 50% depletion of irrigation water which gave a numberof days until 50% flowering 60.79 days. While, in theautumn season, the 75% depletion of of soil moisture gave 40.58 days compared to the level 50% of irrigation water, which gave the highest average of 41.50 days. The increased water shortage may be caused the vital activity within the plant and accelerate of floral branches, the period from of plant and complete the life cycle before exposure to water stress (Ali and Noorka,2013). In autumn season, the same progeny3gave under70% depletion of soil moistureas average 58.8 days to flowering. The number of days to 50% flowering was42.73 and 43.4 days to progeny1 and progeny2 respectively, compared to progeny 3 and original cultivar were gave44.73 days. The difference in flowering time between progenies might be caused by genetics, there are three groups of gene pairs, two of them which interacted with environmental factors that control the response to length and short of photoperiod. The third pairs of genes control the rate of evolution independent of photoperiod, their genes called early genes (Shanko et al,2014).

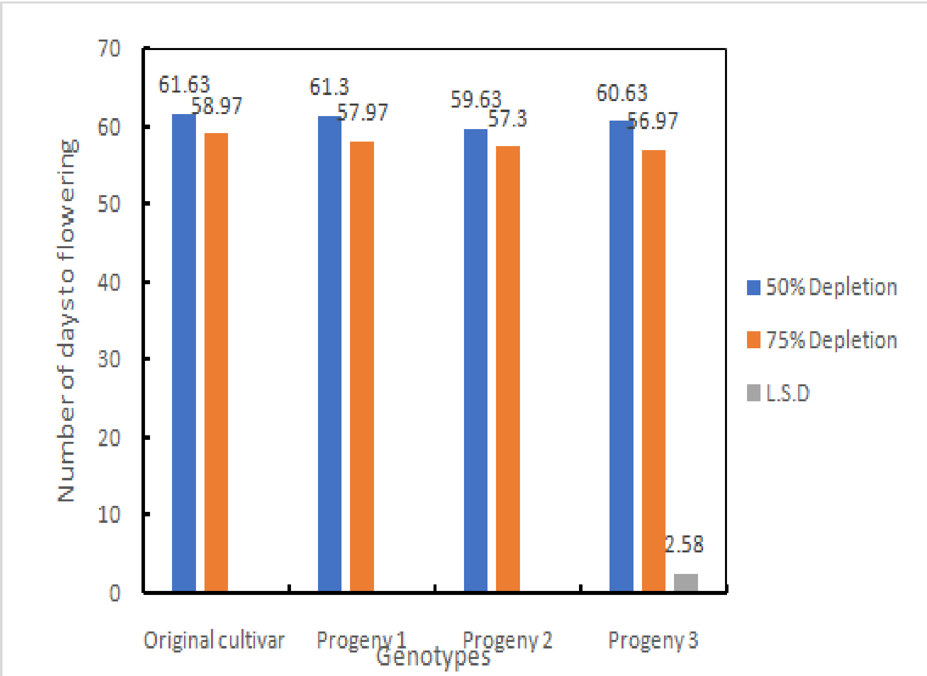

Fig 4. Effect of soil moisture depletion on peroxidase concentration in cowpea progenies in spring 2014.

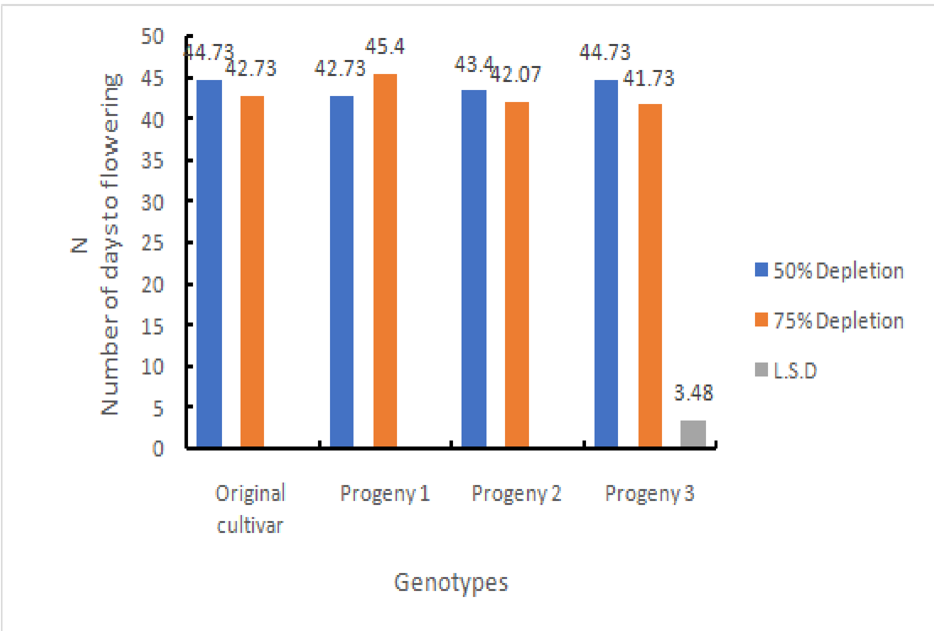

Fig 3. Effect of soil moisture depletion on number of days to flowering in cowpea progenies in spring 2014

The Peroxidase (EC: 1.11.1.7) Activity:

The activity of peroxidase isimportant that acts as an antioxidant of Reactive Oxygen Species (ROS) in plants and this enzyme works to remove the toxicity of free radicals, including H_2O_2 and O^{-2}, which is produced under stress. The results presented in figure 3 showed that there were significant differences between progenies chosen from the selection with the origin cultivar and in both seasons. The selected progeny3 gave in the spring the highest level of 180 units of absorption.gm^{-1} compared with origin cultivar, which gave the lowest mean of 112.2 units of absorption.gm^{-1}. In autumn, the progeny1 gave the highest mean of 184 units absorption.gm^{-1} compared to the rest of genotypes. The selection of a plant to tolerate stress depends on the ability to produce a higheractivity of peroxidase that has the ability to protect plant cells from the damage of ROS (Oraki et al, 2012).

The soil moisture depletion varied in their effect in POD activity, the 50% depletion of soil moisturegave an average of 73.5 absorption units.mg^{-1} compared to 75% Depletion of soil moisture, which gave the highest average of 180.3 absorption units.mg^{-1}. The increasing of peroxidase activity is one of defensive mechanisms after increase of ROS with large quantities in tissue of plant during stress (Sharifi et al, 2012), in which the genes of some plantsstart to encode the antioxidants compounds, including peroxidase which converts H_2O_2 into H_2O and O_2, as well as increasingthe stability of cell membranes and chlorophyll content (Oraki et al, 2012; Mitttler,2002).

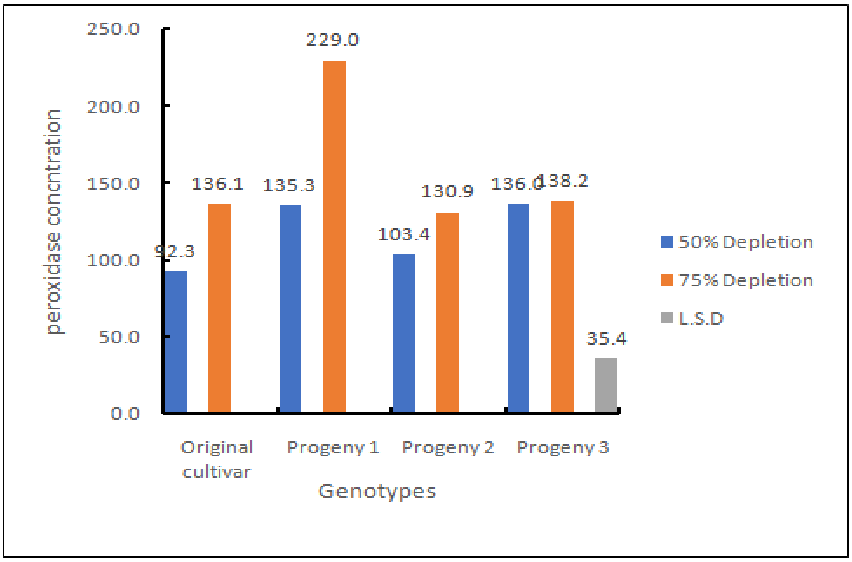

Fig 5. Effect of moisture depletion on peroxidase concentration in cowpea progenies in spring 2014.

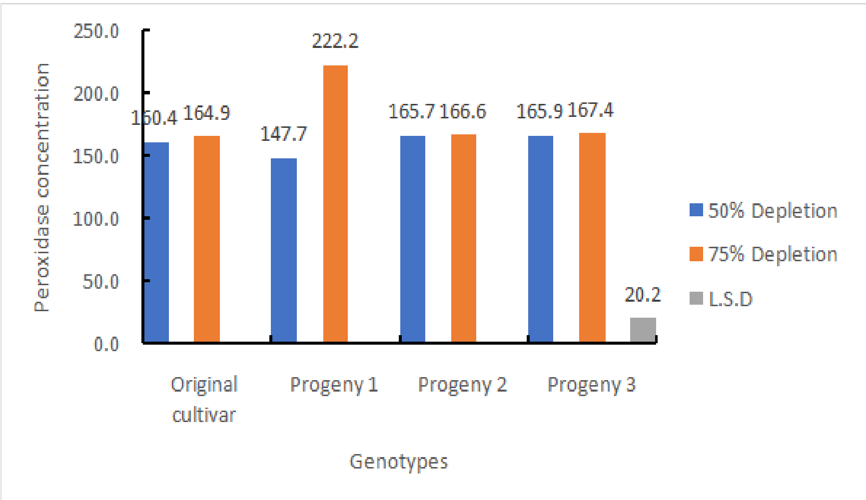

Fig 6. Effect of soil moisture depletion on peroxidase concentration in cowpea progenies in autumn 2014**.**

Proline concentration in leaves:

Proline is an important amino acid with small molecular weights. It's an antioxidant as well as a growth regulator. Prolineisessential in mitigation of stress damage that exposed to plant, especially water stress. The results presented in figure4 showed that there are significant differences between the progenies selected from an origin cultivar. The progeny3 grown in spring season gave the lowest mean of 0.345 mmol gm^{-1} of fresh leave, proline content increased by 36.52%. In autumn, the progeny2 gave the highest average of 0.143 mmol $.gm^{-1}$ of fresh leaves compared to origin cultivar, which gave the lowest average of 0.093 mmol gm^{-1}. The increase of proline content is attributed to the genotype, which is tolerated with stress conditions, which has the inhibitory effect of protein synthesis due to the increase of proteolysis enzymes such as a protease. The results in indicated an increase of the level of amino acid, and reduced osmotic potential of cells to ensure the continuity of water absorption underwater stress conditions, as well as proline, has a role in oxidative stress by maintaining the integrity of the membranes stability (Chinnusamy et al, 2005; parson,1979)

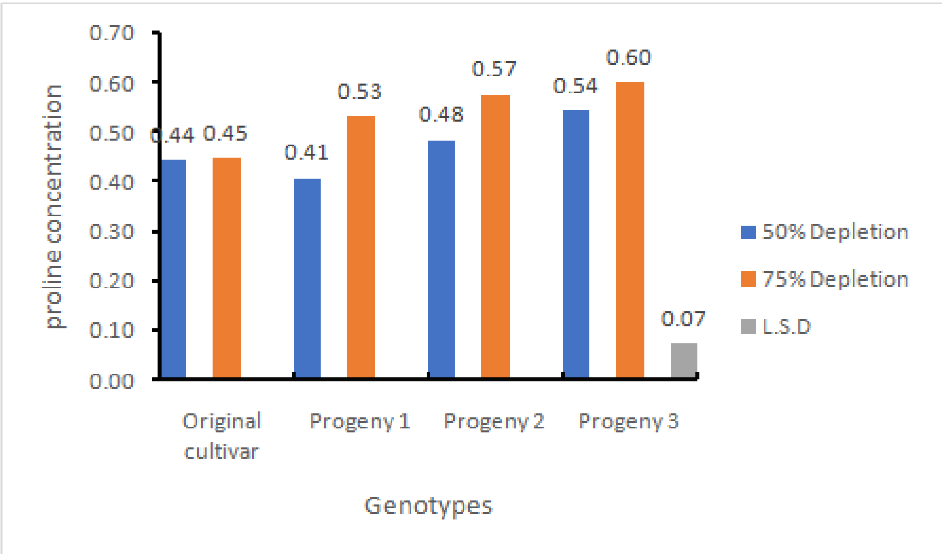

Fig 7. Effect of moisture depletion on proline concentration in cowpea progenies in spring 2014

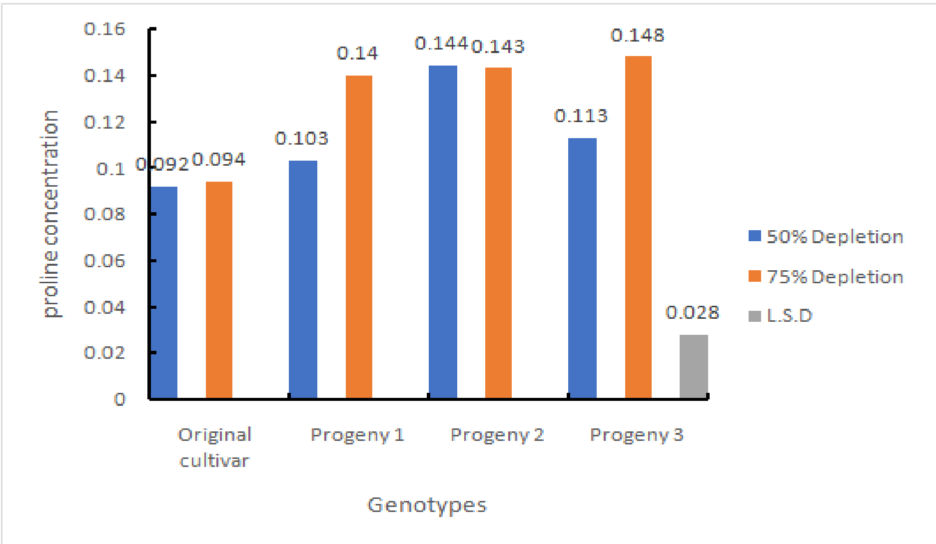

Fig 8. Effect of moisture depletion on proline concentration in cowpea progenies in autumn 2014.

Seed Yield:

Grain yield is the most important field scale, that has been used in the evaluation of cultivars in the fields, in which many genetic and environmental factors contribute toaffecttheyield components by negatively or positively that will be reflected directly on yield (Lchi et al, 2013). The selected progeny3 gave the highest average of 1362.82kg h^{-1} in the spring season at 50% depletion of soil moisture compared to origin cultivar, which gave the lowest average of 1026.27 kg. h^{-1}. while in 75% depletion of soil moisture, the progeny3 gavethehighestaverage of 1129.64kg h^{-1} compared origin cultivar gave the lowest mean of 762.10 kg.h^{-1}. In autumn season (Figure -B) the same progeny3 gave highest mean in both depletion treatments. This reducesthe Biological effectiveness receptively of root mass, low photosynthesis capacity and low balance of plant hormones for all parts of plants (Abed, 2014).

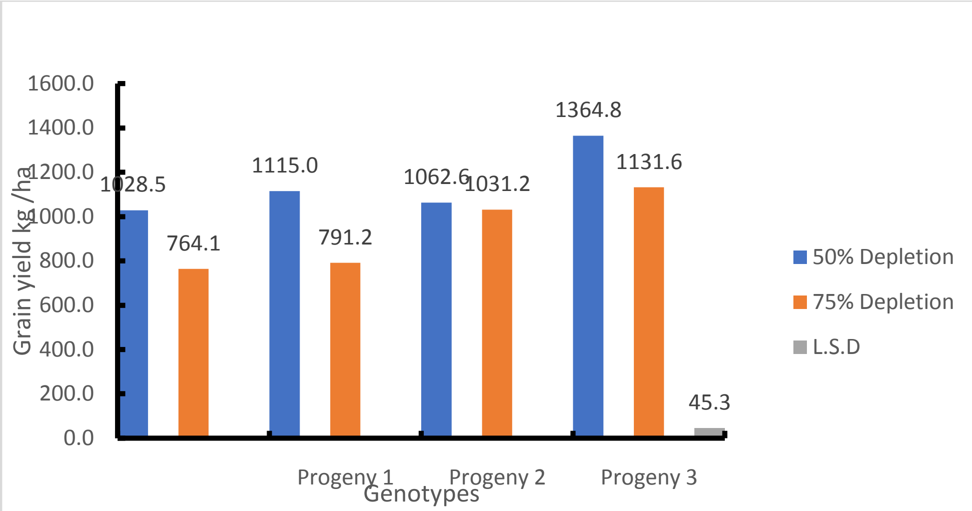

Fig 9. Effect of soil moisture depletion on grain yield in cowpea progenies in spring 2014.

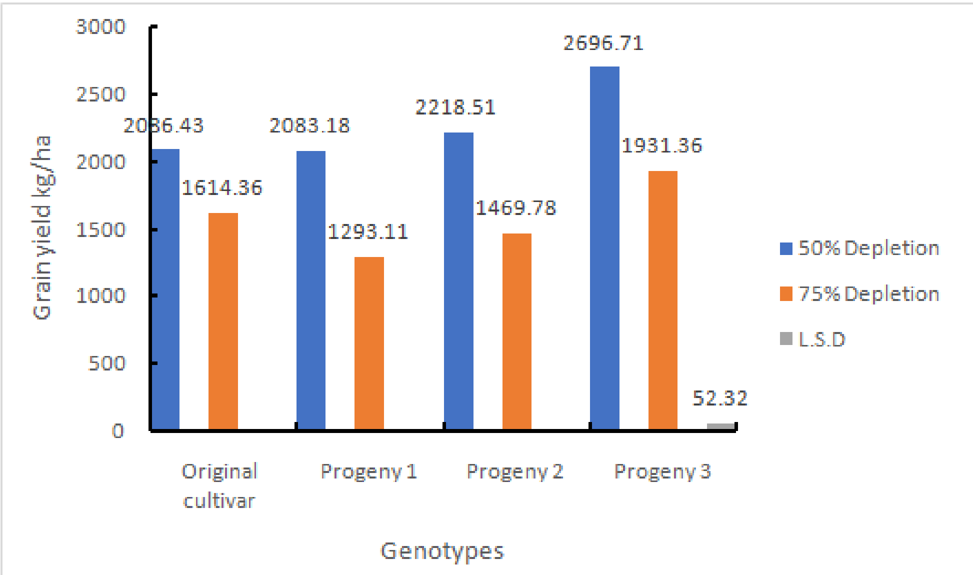

Fig 10. Effect of soil moisture depletion on grain yield in cowpea progenies in autumn 2014.

Water Use Efficiency: (WUE)

Water use efficiency is one of the most effective means of optimizing the investment of water, that controls the number of irrigation frequency and the amount of water supplied in each time. The increasing of WUE and photosynthesis rates lead to the improvement of cropproductivity under water stress (Castro-Nava and Alfredo, 2002). The results presented in figure 5 showed a significant difference between the selected progenies and the origin cultivar for both seasons in WUE, the selected progeny3 under 50% depletion of soil moisture gave anaverageof 0.202 kg seeds.m^3 compared to origin cultivar, which gave anaverageof 0.152 kg seeds.m^3. While in 75% depletion of soil moisture, the same progeny3 gave the highest average of 0.173kg seeds.m3 compared to the origin cultivar that gave the lowest mean of 0.177 kg seeds.m^3. While, in autumn, the progeny3 gave the highest values both 50% and 75%depletion of water Irrigation as average 0.572 and 0.455 kg seeds .m^3 respectively, while the progeny2 gave the lowest mean of 0.296kg seeds.m^3 in 75% depletion of soil moisture. The high WUE of progeny3 is due to increase seed yield and dry matter under stress condition compared to the amount of water used, which is reflected in increased WUE (Oraki et al, 2014).

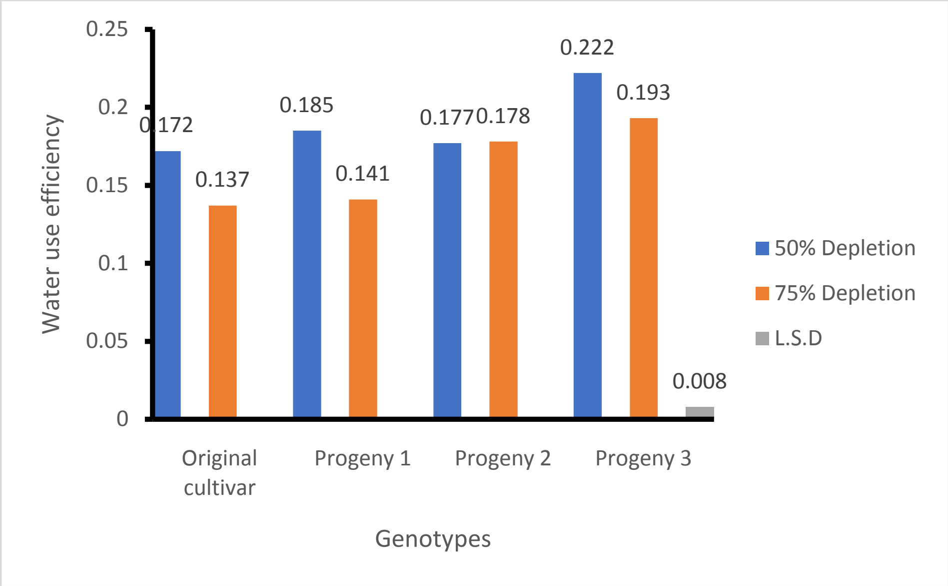

Fig 11. Effect of soil moisture depletion on water use efficiency in cowpea progenies in spring 2014.

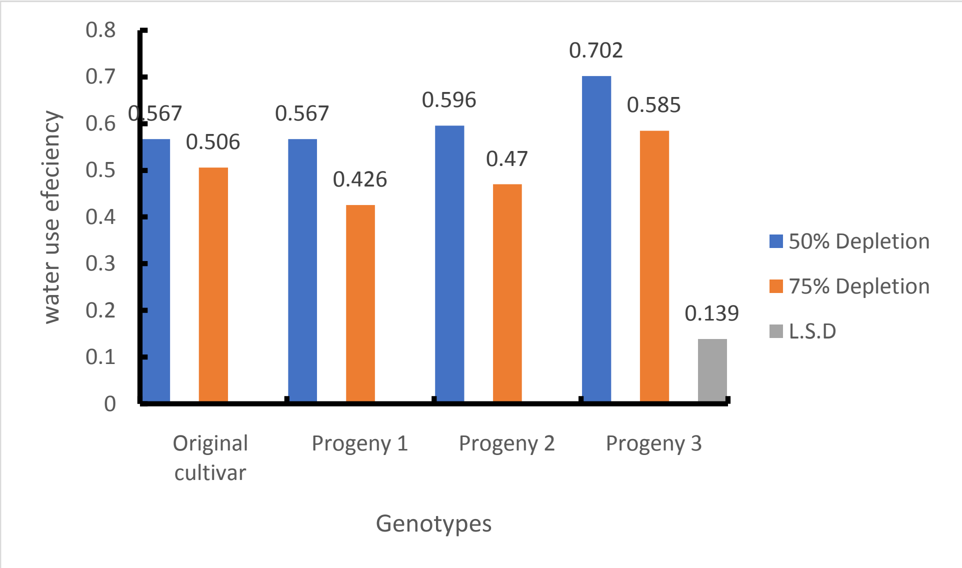

Fig 12. Effect of soil moisture depletion on water use efficiency in cowpea progenies in autumn 2014.

Genetic diversity:

SSR markers have been used to evaluate the genetic diversity and the relationships between the germplasms of cowpea (Hedge and Mishra, 2009).In this study, all the primers used gave amplification of PCR products with (48) bands. SSR primers used to estimate the genetic similarities between the relationships of cowpea germplasms and identified between four selected progenies of cowpea under stress with average (8 main bands) per primer according to (Diouf and Hilu,2005).The number of ranged from (12 bands) to (7 bands) per SSR primer in selected cowpeaprogenies. Six primers generated about (35 bands) polymorphic with average (6) bands per primer among selected progenies of cowpea (Sawadogoet al,2010). This result was agreed with number of studies on soybean (Rongwen et al.,1995). The data in Table (3) shown the genetic distance among all progenies with average between (4.00) to (5.56). High levels of polymorphisms were recorded ranging from primer (3) and (5) with average (75%) and (40%) respectively.

Table 2.the number of main and polymorphic bands and their percentage of polymorphisms across the cowpea progenies and original cultivar

Primers. codes	Sequence **(5'~3')**	Sequence **(3'~5')**	Number of main bands	Number of polymorphic bands	Polymorphism %
SSR-6206	AGGCATGCATTCATCTTTCC	GCAGTCATAACCCCAAAACAA	10	7	70
SSR-6211	TGTCCTCAATTTCAATAACAAGTTT	AACAGTTGGTCGGATACGAAA	7	4	57
SSR-6243	GTAGGGAGTTGGCCACGATA	CAACCGATGTAAAAAGTGGACA	12	9	75
SSR-6257	TGCTTTTGTAAAAGGGTGGAA	ACTTGGACGGAACAGCAGAT	9	6	66
SSR-6281	GCATCAATTTGAGCGAGGAT	GAGTGACATTTCCGCGTCTT	10	4	40
SSR-6291	TCATGAGTTTCCACACACCAA	CCTTCGTATGTATATGTGGCTACTG	9	5	55
Total			48	35	

Table 3. the value of genetic distance between three progenies of cowpea and their origin cultivar.

Genotypes	Origin cultivar	Progeny 1	Progeny 2	Progeny 3
Origin cultivar	0.00	4.06	4.74	5.09
Progeny 1		0.00	4.00	5.56
Progeny 2			0.00	4.58
Progeny 3				0.00

Cluster analysis:

The selected SSR primers have gave significantly differentiated in cowpea progenies. the clustered progenies differently from the morphological classifications (Asar et al,2010). These results showed lower level of similarity between of progenies of cowpea from (Progeny 2)and (Progeny3) .the progenies were different from each to others and from original cultivar . The high level of similarity started from progeny1 and progeny 2 and there are another group of similarity between Progeny3 and Original cultivar . A lot of progenies were clustered depend on the morphological traits and sub-cluster based on the using of the SSR primers . The results in this study different from the others (Oppon-Konadu et al,2005) . who reported lower level of genetic similarity between the genotypes , according to (Dombia et al , 2013).

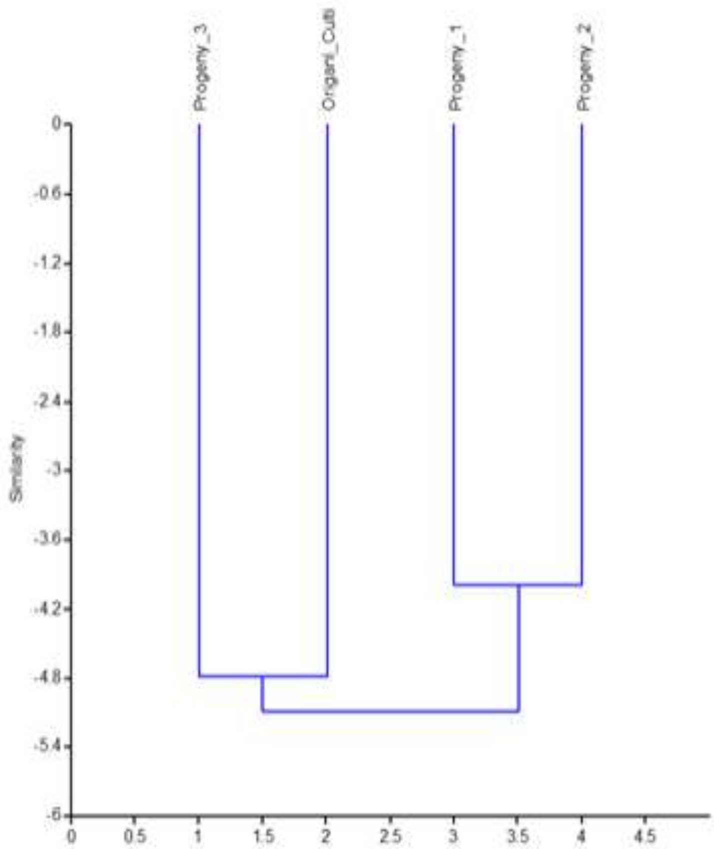

Fig 12. Dendrogram Of Four Germplasms Of Cowpea By Using UPMGA Cluster Analysis Depend On Matrix.

IV. Conclusion

We concluded that the results of PCR-SSR are extremely useful for identifying the genetic distance between three progenies of cowpea and the origin cultivar and the leads to select the progenies that may be production of crosses with good traits such as drought tolerance, high water use efficiency and POD. where this research gives us a insight for the future studies in cowpea programs to produce commercial hybrids with tolerate biotic and abiotic stress.

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