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AGRO-MORPHOLOGICAL CHARACTERIZATION AND GENETIC STUDY OF NEW IMPROVED LINES AND CULTIVARS OF CHICKPEA (*CICER ARIETINUM* L.)

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A B S T R A C T

The genetic resources of chickpea (*Cicer arietinum*, L.) are threatened by the decreasing of its cultivation throughout the world, in most producing countries. So efforts are displayed to alleviate this situation by releasing new well adapted cultivars. The post-harvest data of new chickpea lines and their describing in the different states of agro-morphological development are important and useful in the characterization, evaluation and identification of best lines. This study deals with an agro-morphological characterization of a new genetic material composed of sixteen improved winter chickpea lines which were submitted to a describing with twenty five agro-morphological characters of UPOV's chickpea descriptors (2005). The comparison of the eleven new inbreeding lines with the five witnesses varieties of the collection (Kasseb, Chetoui, Bouchra, Neyer and Beja1) showed that the characters of earliness, tallness and size of grain were improved for all the genetic material and especially for the new lines which were superior to the witnesses varieties (spring of high yield, tolerance to Ascochyta blight and tallness). The two lines 6 and 5 proved to be the best of the collection for most of all studied characters such as precocity, size of pods, size of grains, the duration of the cycle of maturation and in more for number of pods of two grains per plant, number of grains per plant and yield of grain. They have also tall plants, favoring them for a mechanized harvest. These results allow line 6 and line 5 to be selected as lines of the highest capacity of yield, growth and adaptability and can also be used in other genetic programs of improvement.

Keywords: Genetic improvement- Characterization- Agro-morphological characters- heritability - Genetic and phenotypic correlations.

INTRODUCTION

Chickpea (*Cicer arietinum,* L.) is the third most important legume in the world (Singh, 1997), because he has a high nutritional value and a significant role in the valuation in nitrogenous and in phosphor materials of poor grounds (Marcellos, 1984). Chickpea is rich in vegetable proteins, devoid of cholesterol and rich in lysine of which the cereals are deficient. So it constitutes an ideal complement in the meals based on cereals for poor and overcrowded countries such as India or Sub-Saharan Africa (Krishna Murti, 1975).

In spite of the world importance of chickpea in the food safety and his dominating role in the international trade (by reaching eight billions of dollars), his cultivation comes up against numbers of obstacles relative to biotic and non-biotic constraints (Siddique and al, 2000).

Such facts constitute a serious limitation for reaching the potential of the culture and especially a stable production (Singh and Sharma, 2002).

On the other hand, the drought, salinity and the technical level of performances (below than those recommended by the search and popularization), as well as seeds not always selected and handled, are the most spread constraints of producing countries (Remala, 2002).

In Tunisia besides the insufficiency of the culture integration in the SAU (0.2 % which is only dedicated to the chickpea culture), the farmers are often preferring the fallow for the extensive sheep population.

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Furthermore plagues as *Ascochyta* blight and *Fusarium* wilt or roots diseases which are the major biotic constraints whose consequences are an unstable and insufficient production and yield.

In consequence it is necessary to improve the existing genetic material with the release of new varieties with high level of agro-morphological characters and yield, to enhance production and yield.

The first step was to characterize agro-morphologically the sixteen lines, among which eleven are new obtaining and five are cultivated varieties used in this study as witnesses.

MATERIALS AND METHODS

The genetic material is composed of sixteen chickpea lines (table 1), was cultivated in the experimental area of the INRAT (Tunisian Agricultural Research National Institute).

The sowing was done in 29 December 2009 according to a device of a random block in three repetitions. Twenty grains by line have been spread on two meters and half long. Fifty centimeters separates two lines. One irrigation (in March) and two manual kill weeding have been made on the three repetitions.

Table 1. EARPC1 (2009/2010); Chickpea1 yield advanced trial.

	TRT/Line	Origin 2009
1	FLIP97-25C-W5	EARPC1-09 OM (T1)
2	FLIP97-28C-W1	EARPC1-09 OM (T2)
3	FLIP98-22C	EARPC1-09 OM (T7)
4	FLIP98-55C	EARPC1-09 OM (T8)
5	X96TH24-A2-W1-A1-W1-W1-W1	EARPC2-09 OM (T6)
6	X96TH61-A3-W1-A2-W1-A1-W1-W1	Multip OM 09 (V11)
7	X96TH63-A2-A1-A1-A1-A1-W1	EARPC3-09 OM (T2)
8	X97TH85-W1-A1-A2-W1-W2	EARPC3-09 OM (T4)
9	X98TH74-A4-A1-A1-A1	EARPC3-09 OM (T6)
10	X98TH86-W9-W1-A1-A1	EARPC3-09 OM (T7)
11	X2000TH129-A12-W2-A1	EARPC3-09 OM (T10)
12	Kasseb	EARPC3-09 OM (T12)
13	Chétoui	EARPC3-09 OM (T11)
14	Bouchra	MULTIP OM 09
15	Neyer	MULTIP OM 09
16	Béja 1	MULTIP OB 09

List of analyzed characters and their coding: The lines have been observed, noted and measured during the vegetative and reproductive growing states for twenty five agro-morphological characters on ten consecutive plants by line and trial, excepted for the yield in grains that have concerned all plants of the line (twenty plants).

The list of all characters measured is noted on table 2; the method used in describing and notation of the lines is referring to UPOV's (2005) descriptors of chickpea.

Table 2. List of moderate characters.

Character	Code	Number of plants	Unit of measure.
Plant bearing	PP	10	1-3-5
Number of primary branch	RAM1	10	-
Height of plant	PH	10	cm
Coloration of the stem (Anthocyanin)	CA	All the lines (16)	0-1
Color of leaves	CF	5	1-2-3-4-5
Number of leaflets	NFoF	5	-
Length/Width of leaflet	L/laF	5	mm
Flower color	CFl	20	0-1
Pod color	CG	5	-
Length of pod's peduncle	LPG	5	mm
Length of beck pod	LBG	5	mm
Size of pod	TG	5	cm
Duration of emergence	DL	20	days
Appearance date of first sheet	1F	20	days
Appearance date of first pod	1G	20	days
Duration of physiological maturity	DMP	20	days
Number of empty pod per plant	NGVP	10	-
Number of pod of one grain per plant	NG1grP	10	-
Number of pod of two grains per plant	NG2grP	10	-
Number of pod of three grains per plant	NG3grP	10	-
Number of totally pod per plant	NGTP	10	-
Number of grains per plant	NgrP	10	-
Yield in grains per line	-	20	-
Weight of seeds per plant	PoP	10	gram

The rate of proteins was measured according to the method of Kjeldahl. The flour was stemming from finely grinding of seeds resulting from the harvest of chickpea plants on June 2010.

Methodology of analysis: Excepted for the flower color (white for all the lines), anthocyanin tint (absentee in all the lines of the collection) and the seed color (beige for all the lines) which were not subjected to a variance analysis, all the characters measured have been submitted to a variance analysis according to SAS Software version 9,0 with PROC ANOVA procedure LSD option for the mean comparison, the procedure of PROC VARCOMP for the Heritability assessment, the procedure PROC CORR for the study of the correlations and the UPGMA method for the hierarchical classification of lines.

RESULTS

Variance analysis

Variance analysis of agro-morphological characters: The analysis of variance (table 3a and table 3b) shows that the agro-morphological characters used to the description of lines, discriminate significantly to high significantly between the lines; excepted for the number of leaflets by leaf (NFoF), number of pods of three grains by plant (NG3grP), weight of seeds by plant (POP) and for the yield in grains (Rdt) which averages squares show themselves in not significant variance. In consequence these characters are highly influenced by environmental conditions.

On the other hand the characters which are weakly influenced by environment are: Plant height (PH), Number of primary ramification (RAM1), Number of days for the appearance Number of days for physiological maturity (DMP), Number of empty pods by plant (NGVP), number of pods of one grain (NG1grP), number of pods of two grains per plant(NG2grP), number of totally pods per plant (NGTP), number of grains per plant (NgrP) and caliber of grain.

Table 3a. The average squares of morphological and phenological variables: Height of plant (PH), number of primary ramifications (RAM1), number of leaflets by leaf (NFoF), length of pod's peduncle (LPG), length of pod's beak (LBG), size of pod (TG); duration for appearance of first leaf (1F) and duration for appearance of first pod (1G).

S.V	ddl	PH (cm)	RAM1	NFoF	LPG (cm)	LBG (cm)	TG (cm)	1F(j)	1G(j)
Bloc	2	383.345**	0.876**	3.301**	0.368**	0.0002 ns	0.110 ns	38.440**	362.068**
Line	15	140.907**	0.298**	0.444 ns	0.067**	0.007**	6.338**	18.492**	11.170*
Error	30	14.72	0.098	0.36	0.010	0.002	2.13	3.28	4.63
C.V		7.38	8.69	4.34	8.46	18.37	23.26	1.85	1.88
R^2		0.87	0.67	0.54	0.84	0.62	0.59	0.78	0.86

*,** : significant differences at the point of $0.01 < p < 0.05$; respectively, ns: no significant difference at $p < 0.01$.

Table 3b. The average squares of agronomic variables and yield: The duration of physiological maturity (DMP), number of empty pods per plant (NGVP), number of pods of one seed per plant (NG1grP), number of pods of two seeds per plant (NG2grP), number of pods of three seeds per plant (NG3grP), number of total pods per plant (NGTP), count of seeds per plant (NgrP), weight of seeds per plant (POP).

S.V	ddl	DMP	NGVP	NG1grP	NG2grP	NG3grP	NGTP	NgrP	PoP	CAL	Rdt
Bloc	2	181.455**	13.693*	217.363*	114.167**	0.001 ns	846.091**	1287.103**	75.437*	1.207***	7462.71*
Line	15	5.581*	27.078**	211.933**	39.707**	0.014 ns	257.119**	190.376*	23.327ns	0.929***	2469.2ns
Error	30	2.338	2.876	45.501	10.717	0.007	60.041	79.022	14.400	0.085	
C.V		1.202	23.220	18.861	46.110	180.233	15.434	17.740	19.726	3.756	22,62
R^2		0.864	0.833	0.725	0.719	0.491	0.754	0.696	0.536	0.864	0,49

*,** : significant differences at the point of $0.01 < p < 0.05$; respectively, ns: no significant difference at $p < 0.01$.

Table 4: Highest averages value of main agronomical characters analyzed.

Traits	NgrP(gr)	Rdt(gr)	NG2grP(gr)	NGTP	PoP (g)	CAL
Line	6 -15	15 - 6 - 5	6 -5-15	15 - 6	6 - 15	6 – 5 - 15
Values	62 - 54	226 -219	17- 11 – 5	59 - 52	26 - 19	8,3 - 8,5 - 7,6

Genetic study

Heritability: The study of heritability of agro-morphological characters with significant variance and the genotypic and phenotypic correlations between the agro-morphological characters of high heritability and yield of grains, gave the results in tables 5 and 6.

Table 5. Heritability of Agro-morphological characters.

Character	PH	RAM1	LPG	LBG	TG	F	G	DMP	NGVP	NG1grP	NG2grP	NGTP	NgrP	PoP
Heritabiliy	0.740	0.403	0.638	0.457	0.417	0.607	0.319	0.316	0.737	0.549	0.474	0.522	0.319	0.171
Ecart type	0.095	0.160	0.122	0.153	0.157	0.129	0.166	0.166	0.096	0.140	0.152	0.145	0.166	0.165

Genetic and Phenotypic correlations: The study of correlations will permit to identify high heritable characters which are most strongly correlated with yield, and which improvement gave simultaneous improvement of yield.

Table 6 : The genetic and phenotypic correlations between grain yield and agro-morphological characters (height of plant (PH), number of primary branches (RAM1), number of days for appearance of primary flower (1F), size of pod (TG), number of days for maturity (DMP), number of empty pods per plant, number of pods of one grain per plant (NG1grP), number of pods of two grains per plant (NG2grP), number of pods per plant (NGTP)).

Character	PH	RAM1	1F	TG	DMP	NGVP	NG1grP	NG2grP	NGTP
RG	-0.007	0.26	-0.50	0.29	-0.43	0.51	0.58	0.03	0.74
SERG	0.2	0.3	0.2	0.2	0.2	0.2	0.1	0.2	0.1
RP	0.045	0.09	-0.34	0.27	-0.21	0.54	0.57	0.13	0.76
SERP	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.09

(RG) is the genetic coefficient of correlation, (SERG) is its standard deviation, (RP) is the phenotypic coefficient of correlation and (SERG) its standard deviation.

The study of correlations reveals that the characters : Number of totally pods (NGTP) and number of pods of one grain per plant (NG1grP) are the most strongly correlated with yield; (RG= 0.70 and RG = 0.58 respectively).

On the other hands the duration of appearance of primary flower (1F) and physiological maturity (DMP) are strongly but conversely correlated to yield; (RG = -0.58), thus confirming that premature lines in short cycle are the most productive.

The size of pods and the number of primary ramifications are weakly correlated to yield (RG = 0.25).

2.3 Hierarchical classification of lines: The hierarchical classification of lines according to highly heritable agro-morphological characters strongly correlated with yield has given the following dendrogramm (Figure 1).

DISCUSSION

Variance analysis: The comparison of averages with significant variance revealed that; the number of pods of one seed per plant (NG1grP) is the biggest for line 13 and line 6; the number of pods of two seeds per plant (NG2grP) is the biggest for line 6 and line 5 respectively; the number of total pods per plant (NGTP) is the highest respectively for lines 13, 15 and 6. The number of seeds per plant (NgrP) as well as seeds weight per plant (PoP) are highest for line 6 (PoP=26 g ; PoP = 22g for line 5) whereas the weight of witnesses varieties does not exceed the value of 19.6 g in the most favorable case (for Kasseb variety).

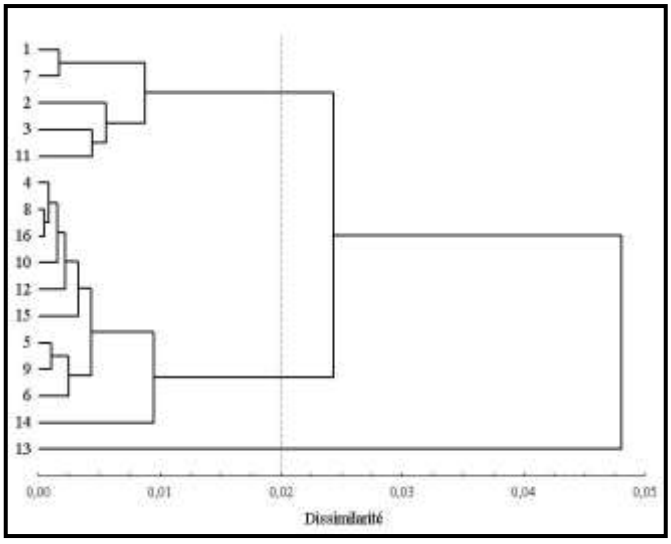

Figure 1. Dendrogramm of lines according to the yield and the agro-morphological characters.

The study shows that among the agronomic characters yield and its components are highly influenced by the environment so the knowledge and determination of his genetic components as well as estimation of environmental effects are important for the choice of

crossing methods, the size of population and intensity of selection.

The number of pods, number of seeds and the size, could be considered as supplementary characters and are genetically controlled by additive effects (Dahliwal and Gil. 1973; Gowda and Bahl. 1978; Malhotra *et al*., 1983.

Concerning the yield the results of our study shows a weight gain of seeds for line 6 in spite of the competition between pods which was exercised. The results of this study shows also that the line15 (Neyer variety) has a score of 226 seeds for the yield whereas line 6 and line 5 have 219 seeds for each one. However the yield per plant is bigger for line 6 (NgrP = 62 grains) than for Neyer variety (the best variety of witnesses in term of yield per plant with NgrP = 52 seeds only). Since yield by plant (number of seeds per plant) is a determining character in the yield estimation, the line 6 is better than Neyer variety for yield per plant because line 6 have best potential of yield. These results are also explained by a better adaptability of Neyer variety because she is resistant to pathogens and let envisaging the practice of later crossings between both lines 15 and 6 and lines 15 and 5.

The results find also a confirmation in the works of Moreno (1985), during his studies of the correlation between the caliber of seeds and the number of seeds by plant. Moreno has found negative correlations at both phenotype and genotype levels, and indicated that the increase of seed caliber cannot end of a consequent improvement of yield. However within the framework of this study, the simultaneous improvement of these characters in priori correlated between them negatively was carried out relatively, because line 6 which is characterized by the biggest caliber of seeds is also on the plan count of total seeds by plant exceeding Neyer variety. Besides the line 6 and line 5 are more successful in yield than the witness varieties (lines 12, 13, 14 and 16) and the line 6 is more favored than lines 12, 14, 15 and 16 for the grain yield per plant. So the practice of crossings between the lines 15 and 6 and 15 and 5 will increase the adaptability of both the lines 6 and 5. The table 4 gives the highest values registered by the lines for main agro-morphological characters analysed (NgrP, Rdt, NG2grP, NGTP, PoP and CAL). The lines 6 and 5 have registered a clear superiority in number of pods of two grains (NG2grP) and weight of grain per plant (PoP) with regard to all the collection.

These profits allowed us to define the lines 6 and 5 respectively as being the most successful of the lot. They are of very advantageous potential yield and endowed with diverse performances relative to big number of characters such as the precociousness, size of pod, number of pods of two grains and weight of grains per plant.

The character height of plants has also benefited of improvement because it varies from 45cm to 65 cm for the new lines while for witnesses varieties it varies from 44 cm to 58. The lines 6 and 5 measure respectively 57cm and 49.5cm joining tall lines.

The highest lines of the collection are line 1 and line 2 with respectively 65cm and 62 cm and are also superior to witnesses varieties and especially to Chétoui variety which is a source of high stature.

A progress is also noted for the character size of pod; indeed the values of lines evolved between 5cm and 8.8 centimeters while the witnesses varieties evolve between 3.2cm (for Chétoui variety) and 6.9 cm (for Neyer variety). Cubero, (1987) has related in his works that the size of pod is not influenced by the environment and varies from 1.5 cm to 3 cm; then we notice the continuous improvement of this character which is attributed to the fact that the plans of selection practiced seem to favor it (he was increasing continuously during these two decades). It could be consequently collected as a relatively reliable criterion of selection. The highest values for the size of the pod are registered by the line 5 with 8.4 cm and the line 6 with 7cm.

Khanna-Chopra and Sinha (1987) , showed that the blooming and the filling of pods are the most sensitive states to the water stress. In consequence the line 5 is endowed with the biggest performance concerning the precocity (duration of appearance of first flower (1F) = 95 days, against 98 days to 104 days for the witnesses varieties); the line 6 records (1F) = 98 days. The precocity registered from most of the obtaining lines varies from 1F = 95 days to 1F = 98 days and seems to favor a potential dodge for water stress which rages at the season end particularly for the Mediterranean climates.

The line 6 and line 5 are more adapted for water deficit and are recommended in seasonal change of various zones. The line 5 is the most premature (1F= 95 days) in comparison with Kasseb variety (1F= 97days).

On the other hands the works of Cubéro, (1987), showed that plants of annual *Cicer* species have a duration cycle from 90 days until 180 days depending on climate conditions. The duration of physiological

maturity registered for all the collection (DMP) is 127 days (on average less than the average noted by Cubero (DMP) = 135 days), constitutes an agronomic advantage and increases the profitability for chickpea culture. And especially for the line 6 and line 5 which cycle duration is respectively 127 and 128 days. The average number of primary branches (RAM1) of new lines is valuing 3.5 and is respectively 3.4 , 3.5 , 3.7 and 4.2 for the lines 6, 15, 5 and 16. This is character has not been improved. The line 1and line 2 are the highest with a raised port (PH = 65 cm and 62 cm respectively), whereas the Chétoui variety which is source of tallness measures 58 cm).

The size of pod is bigger (TG = 8.4 cm and 7 cm respectively for lines 5 and line 6) in comparison with the witnesses varieties which have smaller pods (TG) measured towards 3 cm for Chétoui variety and 6.9 cm for Neyer). The results obtained of all our study permit to select the line 6 and line 5 as the best lines of the collection, because the two lines are combining many performances at the agro-morphological level (in yield of grains (Rdt), size of pods (TG), size of grains (CAL), duration for appearance of first flower (1F), duration for physiological maturity (DMP), number of total grains per plant (NgrP), number of pods of two grains per plant (NG2grP) and in weight of grains per plant (PoP)). Besides the compensation between these characters is reduced to a negligible level within these two lines.

Heritability: The agro-morphological qualitative characters, such as plant height (PH), number of primary branches (RAM1), size of pod (TG), length of pod's peduncle (LPG), length of the pod's beck (LBG), number of empty pods per plant (NGVP), number of days for first flower (1F), number of days for first pod (1G), number of pod of one grain per plant (NG1grP), number of pods of two grains per plant (NG2grP) and number of total pods per plant (NGTP) are highly inherited so they are considered as good criterions of selection ; and are easily (rapidly) improved.

The, number of total grains per plant (NgrP), number of days for the appearance of first pod (1G) and the number of days for physiological maturity (DMP) are of moderate inheritance, so their improvement is slower and demand several generations.

The yield of grains (Rdt), weight of grains per plant (PoP) and protein rate (Pr) have low inheritance; so their improvement is directly difficult. This result shows that these latter are highly dependent of environment.

Correlations: The yield improvement is possible by the improvement of the number of totally pods per plant (NGTP) and number of pods of one grain per plant (NG1grP); and both line 6 and line 5 are recommended to assist such program.

Furthermore the line 6 and line 5 who are tolerant to *Ascochyta* blight will increase their adaptability by crossing them with the line 15 (source of high tolerance to *Ascochyta* blight).

Hierarchical classification: The Figure1 is representing the dendrogramm of lines according to agro-morphological characters for a threshold of dissimilarity of 0.02 and shows that the genetic material studied could be divided into three different groups.

The first group is trained by the line 13, which is spring of *Ascochyta* blight resistance and tallness but is of smallest grains, so it could be identified as a common ancestor.

The second group is trained by two under groups: a first under group established by the line 14 of moderate yields, and the second under group constituted of high yielding lines (with big size grain for new lines and a smallest grain size). This second under group contains the most adapted lines.

The third group contains lines with low yield and moderate size of grains; so he is the group of lines of the lower adaptability.

The line 1 and line 2 which are the highest of the collection by belonging to this third group show that high stature which would be an advantage in terms of harvest mechanization is not perceived as a favorable criterion for high yield.

That property should probably establish an engine of competition between the vegetative and the reproductive growth.

CONCLUSION

The line 6 and the line 5 are endowed with the highest potential of main agro-morphological characters looked for by improvers and that result allows them to be selected as the best lines of the collection.

In more they are high yielding and well adapted to winter cultivation also with an erected bearing and tall type of plants, they can be fate for mechanize harvesting.

The two lines have big size and beige colored grains and then are meeting the requirements of consumers.

The overall combination of a bigger adaptability of lines and cultivation of set up and compact plants with big biological yields are partially reached objectives.

On the other hand, the hierarchical classification reveals a rather wide genetic variability within the lines of the collection which allows to envisage others perspectives of genetic improvement.

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