

Estimate of Heritability and Correlation Analysis for Nitrogen Fixation, Yield and Associated Traits in Chickpea (*Cicer Arietinum L.*)

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ABSTRACT

Chickpea is one of the most cultivated grain legumes in Ethiopia for grain production and amelioration of soil fertility through N-fixation, and income generation. This study was conducted to estimate the heritability and correlation analysis of N-fixation in chickpea. Six F₁ crosses were evaluated in lath house during the year 2014/15 using randomized complete block design (RCBD) with two replications at Debre Zeit Research Center. Heritability of the characters was estimated in F₁ generations from the combining ability analysis of a di-allele cross using fixed model expectations of mean squares for GCA and SCA. The calculated narrow sense heritability was high (>0.50) for all traits except for seed filling duration, number of pod per plant, shoot nitrogen fixation, grain harvesting index and grain production efficiency. All symbiotic traits such as number of nodules, shoot nitrogen fixation, N fixed in biomass, N fixed in grain, Nitrogen harvest index (NHI), Grain N yield, Shoot N yield, Biomass N yield, Nodule dry weight, Shoot and grain protein contents and Nitrogen showed strong positive association with yield and other agronomic traits, indicating the improvement of symbiotic traits results in the improvement of other agronomic traits of the crop. The prevailing high heritability indicates the possibility of improving the traits either by selection or crossing of selected parents. This study is first of its kind in Ethiopia, and helps the future breeding and genetics in chickpea dealing with the nitrogen fixation processes.

Key words: Correlation, Heritability; N-fixation, nodulation,

1. INTRODUCTION

Chickpea can fix up to 140 kg N ha⁻¹ from air, consequently, it meets 80% of its nitrogen (N) requirement from symbiotic nitrogen fixation. Moreover, it leaves substantial amount of residual nitrogen for subsequent crops and adds plenty of organic matter to maintain and improve soil health and fertility (Gaur *et al.*, 2010). Therefore, breeding of chickpea genotypes for efficient symbiotic nitrogen fixation can be considered as one of the alternatives to maximize/realize the benefits of the biological nitrogen fixation process. Biological nitrogen fixation (BNF) is a sustainable alternative for nitrogen supply to agriculture worldwide. One approach to increasing BNF in agriculture is to breed and use legumes with greater BNF capacity (Biabani *et al.*, 2011). The phenomenon of biological nitrogen fixation is made possible by the association of soil bacteria (functionally regarded as rhizobia) and the host plant (belonging to the family leguminosae) for their mutual benefit. Hailemariam *et al.*, (2006) reported that efforts were made to enhance nitrogen fixation through selection of effective and competitive strains of Rhizobia and competent strains were identified in Ethiopia. However, development of better strains alone may not provide the expected response without compatible host genotypes (Chen *et al.*, 2003), as the amount of nitrogen fixed is determined by the gene present in the bacteria and the host (Ali *et al.*, 2000). The genetic potential for the improvement of symbiotic nitrogen fixation in legumes was reported, but the possibilities for improving host-strain symbiotic efficiency through breeding is limited (Hardarson, 2004) Rengel (2002) reported nodulation in the rhizobia–legume symbiosis may be limited by an insufficient amount of the nod-gene inducers released from seed and/or roots. Furthermore, increased efficiency of symbiotic nitrogen fixation can be achieved by selecting not only the best host genotypes but by selecting the best combination of host genotype and nodule bacteria (Rengel, 2002). However, the role of host plants attracted little attention in Ethiopia (Geletu *et al.*, 2004, Gemechu *et al.*, 2012). A lot of exploratory works remains the subject of future investigation in understanding the genetics of efficient fixation of nitrogen in chickpea and identification of the best genotypes. Therefore, designed efforts to breed yield responding genotypes for improved nitrogen fixation through rhizobial symbiosis will contribute toward sustainability of agricultural ecosystems in which soil-plant-microbe interactions will be

better exploited. The objectives of this study were designed to estimate the heritability and correlation analysis of N-fixation in chickpea.

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2. MATERIALS AND METHODS

2.1. Site Description

Generation of progeny was carried out at Debre Zeit Agricultural Research Center (DZARC) wire house crossing block during the cropping season of 2013/2014. The center is located at an altitude of 1900m.a.s.l, with annual temperature of (max=26.1°C) and (min=8°C). The annual rainfall in the experimental area is 850mm. Its soil type is vertisol with pH of 7.45.

2.2. Experimental Materials and Design

Two nodulating chickpea (ICC5003 and ICC19180) and two non-nodulating (ICC4918 and ICC19181) genotypes (Table 1) were introduced from ICRISAT and inter-crossed to develop F₁ generations using hand emasculation pollination techniques. The four parental materials with their six crosses (F₁) and two recently released standard checks (Arerti and Natoli, which are moderately nodulated) were planted in Randomized Complete Block Design with 2 replication using irrigation during 2014. Plant to plant and row to row distance was 10 and 30cm, respectively. Each plots consisted of 4 rows of 2 meters long. Two seeds per hill were planted and finally thinned out to one plant per hill after 3 weeks of emergence. All other agronomic practice like weeding and irrigation was done as per recommendation.

Table 1. Phenotypes, nodulation characteristics and origin of the chickpea genotypes.

No	Genotype	Phenotype	Nodulation Characteristics	Origin
1	ICC5003	Desi	Nod ⁺	ICRISAT

2	ICC19180	Kabuli	Nod ⁺	ICRISAT
3	ICC4918	Desi	Nod ⁻	ICRISAT
4	ICC19181	Desi	Nod ⁻	ICRISAT
5	Arerti	Kabuli	Nod ⁺	DZARC
6	Natoli	Desi	Nod ⁺	DZARC

Nod⁺= nodulated, Nod⁻= non-nodulated

Data was collected on days to 50% flowering, Days to 90% maturity, Grain production efficiency, Biomass production rate, Economic growth rate, Number of pods per plant, Biomass yield, Grain yield, Grain Harvest index, 100 seed weight, Shoot dry weight at physiological maturity, Shoot dry weight ratio (based on Bioversity International 2007, IBPGR (1985)) guidelines for crop descriptors). Number of nodules, Shoot nitrogen fixation, N fixed in biomass , N fixed in grain, Nitrogen harvest index (NHI), Grain N yield, Shoot N yield, Biomass N yield, Nodule dry weight , Shoot and grain protein contents according to Gemechu *et al.*, (2013). Nitrogen analysis was done according to Kjeldahl procedure (AOAC, 1970). Estimation of heritability and correlation were done according to Bulmer (1980) cited in Hasanuzzaman *et al.*(2012) and Mutengwa. *et al.*(2012)

Heritability of the characters was estimated in F₁ generations from the combining ability analysis of a diallel cross using fixed model expectations of mean squares for general combining ability (GCA) and specific combining ability (SCA)(Hasanuzzaman *et al.*, 2012). Heritability estimates were grouped as high (>50%, moderate (20%-50%) and low (<20%) as suggested by Stansfield (1986).

3. RESULTS AND DISCUSSION

3.1. Heritability Estimates

Variation in narrow sense heritability was observed among the studied traits (Table 2). High narrow sense heritability value was recorded for days to 50% flowering, days to 90% maturity, number of nodule, nitrogen fixed in biomass and grain, nitrogen yield in grain, shoot and biomass, biomass and grain yield, 100 seed weight, shoot dry weight ratio, nodule dry weight, seed protein content, grain protein content, biomass production rate and economic growth rate. For seed filling duration, shoot nitrogen fixation, nitrogen harvest index and shoot dry weight at physiological maturity narrow sense heritability was moderate, whereas low narrow sense heritability was recorded for number of pods per plant, nitrogen harvest index, and grain harvest index and grain production efficiency (Table 2).

In traits with high narrow sense heritability, the inheritance of the traits controlled by additive gene action whereas for those with low narrow sense heritability the inheritance of traits is controlled by non-additive gene action***. Except for number of pods per plant, nitrogen harvest index, grain harvest index and grain production efficiency, the other all studied traits seemed to be controlled by additive gene action. In a genetic study of agronomic traits, Rohman *et al.* (2003) reported that 100 seed weight, seed yield per plant and days to flowering were governed to a greater extent by additive gene effect in mungbean. Sukhumaporn *et al.*(???) found the largest narrow sense heritability estimate for days to 50% flowering (88%), pods per plant (58.4%), hundred seed weight (87%) and grain yield (63.1%) in mungbean. Hasanuzzaman *et al.* (2012) was also observed and recorded the highest narrow sense heritability for days to 50% flowering (61.82%) and the least for grain yield per plant (18.42%) in chilli crop. The result from Sukhumaporn (??) is in agreement with the estimate made for the chickpea genotypes in the present study for grain yield and days to flowering. Khattak *et al.* (2002) reported the genetic effect of dominance variance for the number of pods per plant and the number of seeds per pod in mung bean. In cowpea nodule weight and nodule number were controlled by both additive and non-additive gene action (Singh *et al.*, 1985).

The choice of breeding methods for the improvement of nitrogen fixation is influenced by the complex nature of gene expression for nitrogen fixation; trait-heritability; and the destructive nature of many methods for estimating N₂-fixation (Ali *et al.*, 2000). Hence, selection for traits with high narrow sense heritability estimates would be effective.

Table 2. Heritability of symbiotic and agronomic traits

	NN	NDW	SNY	SNF	NFB	NFG	GNY	BNY	NHI	SPC	GPC	h ² %
DF	0.001 ^{ns}	-0.53*	-0.11 ^{ns}	-0.09 ^{ns}	-0.15 ^{ns}	-0.24 ^{ns}	-0.14 ^{ns}	-0.17 ^{ns}	-0.10 ^{ns}	-0.11 ^{ns}	-0.21 ^{ns}	62
DM	-0.24 ^{ns}	-0.47*	-0.25 ^{ns}	-0.18 ^{ns}	-0.29 ^{ns}	-0.44 ^{ns}	-0.39 ^{ns}	-0.33 ^{ns}	-0.21 ^{ns}	-0.20 ^{ns}	-0.43*	96
SFD	-0.27 ^{ns}	0.40*	-0.08 ^{ns}	-0.03 ^{ns}	-0.05 ^{ns}	-0.06 ^{ns}	-0.18 ^{ns}	-0.06 ^{ns}	-0.06 ^{ns}	-0.02 ^{ns}	-0.11 ^{ns}	36
NPP	0.35 ^{ns}	0.07 ^{ns}	0.23 ^{ns}	0.35 ^{ns}	0.43*	0.50*	0.78**	0.28 ^{ns}	0.80**	0.30 ^{ns}	0.52*	6
BM	0.77**	0.40*	0.93**	0.51*	0.57*	0.60*	0.52*	0.92**	-0.18 ^{ns}	0.56*	0.60*	94
GY	0.66*	0.10 ^{ns}	0.43*	0.42*	0.50*	0.55*	0.95**	0.50*	0.78**	0.41*	0.60*	71
GHI	0.13 ^{ns}	-0.18 ^{ns}	-0.19 ^{ns}	0.13 ^{ns}	0.14 ^{ns}	0.12 ^{ns}	0.60*	-0.11 ^{ns}	0.95**	0.07 ^{ns}	0.16 ^{ns}	7
HSW	0.58*	0.20 ^{ns}	0.26 ^{ns}	0.36 ^{ns}	0.40 ^{ns}	0.41*	0.66*	0.36 ^{ns}	0.45*	0.40 ^{ns}	0.47*	67
SDWP	-0.09 ^{ns}	-0.05 ^{ns}	0.10 ^{ns}	-0.02 ^{ns}	-0.12 ^{ns}	-0.26 ^{ns}	-0.30 ^{ns}	0.02 ^{ns}	-0.35 ^{ns}	-0.04 ^{ns}	-0.31 ^{ns}	46
SDWR	-0.09 ^{ns}	-0.05 ^{ns}	0.10 ^{ns}	-0.02 ^{ns}	-0.12 ^{ns}	-0.26 ^{ns}	-0.30 ^{ns}	0.02 ^{ns}	-0.35 ^{ns}	-0.04 ^{ns}	-0.31 ^{ns}	70
GPE	-0.17 ^{ns}	0.56*	0.001 ^{ns}	0.06 ^{ns}	0.07 ^{ns}	0.10 ^{ns}	-0.04 ^{ns}	0.044 ^{ns}	-0.02 ^{ns}	0.07 ^{ns}	0.05 ^{ns}	13
BPR	0.75**	0.45*	0.90**	0.50*	0.57*	0.63*	0.55*	0.90**	-0.13 ^{ns}	0.55*	0.62*	96
EGR	0.70*	0.03 ^{ns}	0.43*	0.42*	0.50*	0.55*	0.95**	0.50*	0.75**	0.41*	0.61*	65
h ² %	94	53	96	25	66	87	81	98	17	85	91	

*=significant difference at 5% level of probability, **= highly significant difference at 5% level of probability ns= no significance difference BM=biomass yield, BNY=biomass nitrogen yield, BPE= biomass production efficiency, DF=days to 50% flowering, DM=days to 90% maturity EGR= economic growth rate, GHI=grain harvest Index, GPC=grain protein content, GPE=grain production efficiency, GNY=grain nitrogen yield, GY=grain yield, HSW=hundred seed weight, h²=narrow sense heritability NDW=nodule dry weight, NFB=nitrogen fixed in biomass, NFG=nitrogen fixed in grain, NHI=nitrogen harvest index, NN=number of nodule, NPP=number of pods per plant, SDWP=shoot dry weight at physiological maturity, SDWR= shoot dry weight ration, SFD=seed filling duration, SNF=shoot nitrogen fixation, SNY=shoot nitrogen yield, SPC=shoot protein content.

3.2. Traits Association

Significant and positive association were recorded among all symbiotic traits studied except for nodule dry weight and nitrogen harvest index, number of nodule and grain nitrogen yield. Except with grain nitrogen yield ($r=0.67$), nitrogen harvest index also showed no significant association with all symbiotic traits (Table 3). The association between the other traits were high and positive ($r=0.52-0.99$) (Table 3). Similar results were reported for most of the symbiotic traits by Gemechu *et al.* (2013).

High positive correlation was reported by Singh *et al.*, (1985) in cowpea among the following traits including nodule number, nodule volume and nodule dry weight. However, Gemechu *et al.*, (2013) found non-significant correlation between grain nitrogen yield and number of nodule, nodule dry weight, shoot nitrogen content, and shoot nitrogen fixation. The same author reported positive and significant association of nitrogen harvest index with grain nitrogen yield but negative and significant association with number of nodule, shoot nitrogen content, shoot nitrogen fixation, biomass nitrogen content and shoot nitrogen yield in chickpea genotypes. In contrary to this correlation values, positive and highly significant associations were observed among grain nitrogen yield and biomass nitrogen yield (Gemechu, *et al.*, 2013). The highest correlation was recorded among the following traits such as shoot nitrogen fixation and shoot protein content, nitrogen fixed in grain and grain protein content, biomass nitrogen yield and shoot nitrogen yield, nitrogen fixed in biomass and shoot nitrogen fixation, nitrogen fixed in biomass and shoot protein content ($r=0.97-0.99$) (Table 3). Highly significant positive correlation was reported in peanut for shoot dry weight, nodule dry weight and nitrogen fixation (Sikinarum. *et al.* 2007). In other investigation, positive association among total nitrogen content, total fixed nitrogen content, nodule dry weight and shoot dry weight over location was reported in peanut (Dinh *et al.*, 2013). This implies the increase or decrease of one of the traits affect the other traits in the direction they associated.

Grain yield was positively associated with number of pods per plant ($r=0.83$), grain harvest index ($r=0.74$), 100 seed weight ($r=0.62$), economic growth rate ($r=0.97$), biomass production rate ($r=0.44$) and with all of the symbiotic parameters studied except nodule dry weight. In

addition, days to 90% maturity and days to 50% flowering ($r=0.85$), grain production efficiency and seed filling duration ($r=0.93$), 100 seed weight and biomass yield ($r = 0.49$), 100 seed weight and grain yield ($r=0.62$), biomass production rate and 100 seed weight ($r = 0.53$), economic growth rate and 100seed weight($r = 0.66$), shoot dry weight ratio and shoot dry weight at maturity, ($r=1$), biomass production rate and biomass yield ($r = 99$), shoot dry weight at maturity and days to 90% maturity ($r = 67$) and shoot dry weight ratio and days to maturity were positively correlated (Table 3).

Seed filling duration and days to 50% flowering ($r = -0.79$), grain production efficiency and days to 50% flowering($r=-0.93$), grain production efficiency and days to 90% maturity ($r=-62$), biomass yield and days to 90% maturity ($r=-0.53$), biomass production rate and days to 90% maturity($r=-0.51$), grain harvest index and biomass yield($r=-0.74$), biomass production rate and grain harvest index ($r = -0.62$), biomass production rate and shoot dry weight at maturity ($r = -0.54$) and biomass production rate and shoot dry weight ratio ($r=-0.54$) were negatively correlated. The rest of all the character did not exhibit significant correlation (Table 3). Gemechu *et al.*, (2013) recorded positive and significant association of grain yield and seed filling period, number of pods, shoot dry weight at maturity, biomass yield, harvest index, grain production efficiency, biomass production rate, and economic growth rate in the studied chickpea genotypes but negative association for grain yield with flowering and maturity time. Geletu and Ketema, (2003) reported positive correlation among yield and biological yield, days to 50% flowering and 100 seed weight in chickpea. The same author reported significant correlations of biological yield with grain yield, days to 50% flowering, days to 90% maturity, number of nod per plant, 100 seed weight, number of seed per plant, and harvest index.

In the current study, except nodule dry weight and shoot nitrogen yield all symbiotic traits were positively correlated with grain yield ($r=0.41-0.95$). Except nitrogen harvest index, symbiotic traits revealed positive association with biomass yield ($r=0.40-0.93$). Except with nodule dry weight, shoot nitrogen yield, shoot nitrogen fixation, nitrogen fixed in biomass, nitrogen harvest index and shoot protein content most symbiotic traits were also positively associated with 100 seed weight ($r=0.41-0.66$). Characters correlated positively with grain

yield were also positively correlated with each other (Table 3). So selection based on symbiotic traits, which are positively related to grain yield, will help in identifying genotypes with high yielding potential.

Nodule number was positively correlated with biomass yield, grain yield, 100seed weight, biomass production rate and economic growth rate. However, significant association was not observed with the rest of agronomic characters. Nodule dry weight revealed positive association with seed filling duration, biomass yield and grain production efficiency, biomass production rate, but negatively associated with days to 50% flowering and days to 90% maturity. Positive association was observed among shoot nitrogen yield, biomass yield, biomass production rate and economic growth rate, but neither negatively nor positively correlated with other agronomic traits (Table 3). Positive and high correlation between nodule number and yield and other yield components were reported by different authors in chickpea (Asfaw and Angaw 2003)

Nitrogen fixed in grain, shoot, and biomass, grain nitrogen yield, biomass nitrogen yield, shoot protein content, grain protein content, showed positive and significant correlations with biomass yield, grain yield, biomass production rate and economic growth rate. However, associations among the other traits were found to be negative (Table 3). The same results from similar undertakings were reported by Asfaw and Angaw (2003). Hence, grain yield and nitrogen yield parameters could be used to select effective genotypes for nitrogen fixation. So, it is difficult to separate N-fixation from yield experiment (Keyser and Fudi, 1992).

Table 3. Correlation coefficient(r) between symbiotic and agronomic traits

	DF	DM	SFD	NPP	BM	GY	GHI	HSW	SDWP	SDWR	GPE	BPR	EGR
DF	1												
DM	0.84**	1											
SFD	-0.79**	-0.34 ^{ns}	1										
NPP	-0.19 ^{ns}	-0.30 ^{ns}	-0.0008 ^{ns}	1									
BM	-0.21 ^{ns}	-0.38 ^{ns}	-0.06 ^{ns}	0.17 ^{ns}	1								
GY	-0.15 ^{ns}	-0.35 ^{ns}	-0.12 ^{ns}	0.83**	0.43*	1							
GHI	-0.03 ^{ns}	-0.09 ^{ns}	-0.03 ^{ns}	0.76**	-0.24 ^{ns}	0.74**	1						
HSW	-0.02 ^{ns}	-0.21 ^{ns}	-0.20 ^{ns}	0.26 ^{ns}	0.25 ^{ns}	0.62*	0.43*	1					
SDWP	0.26 ^{ns}	0.54*	0.13 ^{ns}	-0.22 ^{ns}	0.07 ^{ns}	-0.22 ^{ns}	-0.23 ^{ns}	-0.37 ^{ns}	1				
SDWR	0.26 ^{ns}	0.54*	0.13 ^{ns}	-0.22 ^{ns}	0.07 ^{ns}	-0.22 ^{ns}	-0.23 ^{ns}	-0.37 ^{ns}	1.00**	1			
GPE	-0.93**	-0.62*	0.92**	0.05 ^{ns}	0.04 ^{ns}	-0.03 ^{ns}	-0.03 ^{ns}	-0.03 ^{ns}	-0.14 ^{ns}	-0.14 ^{ns}	1		
BPR	-0.32 ^{ns}	-0.51*	-0.007 ^{ns}	0.20 ^{ns}	0.98**	0.46*	-0.21 ^{ns}	0.29 ^{ns}	-0.02 ^{ns}	-0.02 ^{ns}	0.14 ^{ns}	1	
EGR	0.001 ^{ns}	-0.28 ^{ns}	-0.32 ^{ns}	0.79**	0.43*	0.97**	0.70*	0.65*	-0.28 ^{ns}	-0.28 ^{ns}	-0.20 ^{ns}	0.45*	1

*=significant difference at 5% level of probability, BM=biomass yield, BPR= biomass production rate ,DF=days to 50% flowering, DM=days to 90% flowering, EGR= economic growth rate, GHI=grain harvest index, GPE=grain production efficiency, GY=grain yield, HSW= hundred seed weight, NPP=number of pods per plant, ns= no significance difference, SFD=seed filling duration, SDWP=shoot dry weight at physiological maturity, SDWR= shoot dry weight ratio

Table 3. Continued

	NN	NDW	SNY	SNF	NFB	NFG	GNY	BNY	NHI	SPC	GPC
NN	1										
NDW	0.32 ^{ns}	1									
SNY	0.80**	0.49*	1								
SNF	0.64*	0.68*	0.72**	1							
NFB	0.69*	0.71**	0.75**	0.97**	1						
NFG	0.70*	0.70*	0.73**	0.84**	0.94**	1					
GNY	0.76**	0.26 ^{ns}	0.55*	0.55*	0.65*	0.73**	1				
BNY	0.85**	0.57*	0.98**	0.78**	0.82**	0.81**	0.63*	1			
NHI	0.17 ^{ns}	-0.18 ^{ns}	-0.17 ^{ns}	0.04 ^{ns}	0.11 ^{ns}	0.19 ^{ns}	0.67*	-0.09 ^{ns}	1		
SPC	0.68*	0.69*	0.76**	0.98**	0.97**	0.85**	0.55*	0.82**	0.001 ^{ns}	1	
GPC	0.72**	0.62*	0.71**	0.80**	0.91**	0.98**	0.79**	0.80**	0.24 ^{ns}	0.81**	1

*=significant difference at 5% level of probability, BNY=biomass nitrogen yield, GNY=grain nitrogen yield, GPC=grain protein content , NDW=nodule dry weight, NFB=nitrogen fixed in grain, NFG=nitrogen fixed in grain, NHI=nitrogen harvest index, NN=number of nodule, ns= no significance difference , SNF=shoot nitrogen fixation, SNY=shoot nitrogen yield, SPC=shoot protein content,.

5. CONCLUSION

The highest narrow sense heritability value was estimated for days to 50% flowering, days to 90% maturity, number of nodule, nitrogen fixed in biomass, nitrogen fixed in grain, grain nitrogen yield, shoot nitrogen yield, biomass nitrogen yield, biomass yield, grain yield, 100seed weight, shoot dry weight ratio, nodule dry weight, seed protein content, grain protein content, biomass production rate and economic growth rate, and were found to be high. Moderate heritability was recorded for seed filling duration and shoot nitrogen fixation, whereas, the lowest heritability was obtained from number of pods per plant and grain production efficiency indicating the significant role of non-additive gene action in controlling the inheritance of these traits. Results of this study demonstrated the importance of additive gene action in the inheritance of some of agronomic and symbiotic traits. Since all of the symbiotic traits showed positive and significant correlation with grain yield, biomass yield and 100 seed weight, selection for symbiotic traits in chickpea help in improving the yield of the crop. Indeed the result of this study could be an indicator to recognize the promising genotypes to be exploited either as F₁ hybrids or as a resource population for further selection.

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