

Diallel analysis of common bean for secondary metabolites biosynthesis under salt stress.

Université des
Constantine I

Torche Yacine

Diallel analysis for secondary metabolites biosynthesis in common bean (*Phaseolus vulgaris* L.) under salt stress.

Summary: To evaluate secondary metabolites productivity of common bean under salt stress, four varieties growing in Algeria have been selected. A half-diallel cross has been carried out producing six hybrids, the population was growing in salt conditions: 0, 50 and 100mM NaCl in a complete randomized block design. The total phenolic and the total flavonoid content have been evaluated and diallel analysis has been conducted to assess the best hybrids for yield and polyphenolic biosynthesis. The results revealed high significant genotypic variations between the hybrids and their parents. Both general and specific combining abilities were highly significant, revealing the important role of both additive and dominant gene effects in the inheritance of these traits.

Keywords: Common bean, Diallel analysis, Secondary metabolites, Salt stress.

ملخص: لتقييم إنتاج الأيضات الثانوية لنبات الفاصولياء تحت ظروف الإجهاد الملحي، تم اختيار أربعة أصناف تنمو في الجزائر. خضعت الأصناف الأبوية إلى تجين نصف أليلي أدى إلى إنتاج ستة هجن، زرعت الهجن والآباء تحت ظروف ملحية باستخدام ثلاثة تراكيز: 0، 50 و 100Mm من كلوريد الصوديوم وفق تصميم الكتل العشوائية الكاملة. تم تقييم إجمالي الفينول ومحتوى الفلافونويد الكلي لجميع الانماط الجينية ثم خضعت النتائج البيوكيميائية لتحليل diallel وذلك لتقييم أفضل الهجن لإنتاج الأيضات الثانوية وكذا المردودية. كشفت النتائج اختلافات معنوية كبيرة بين الهجن والآباء، وأن القدرات العامة والخاصة على التوافق لهذه التراكيب الوراثية كانت جد معنوية لجميع المعايير المدروسة تحت الإجهاد الملحي، كما أسفرت النتائج على أهمية كل من الفعل الوراثي التراكمي واللاتراكمي في توريث الصفات المدروسة.

الكلمات المفتاح: الفاصولياء، تحليل أليلي، الأيضات الثانوية، إجهاد الملحي.

Preface:

Salinity is one of the major abiotic stresses factors limiting plant growth, metabolism and productivity¹. In the arid and semiarid areas, salinity is mainly caused by the irrigation water which contains considerable amounts of soluble salts; the accumulation of salts into the top layer of the soil causing by over-irrigation; proximity to the sea and the capillarity rise of salts from underground water into the root zone due to excessive evaporation. In addition, high evaporation rate, poor water management and low rainfall could increase salinity levels in these areas².

Salinity affects almost every aspects of the physiology and the biochemistry of plants and significantly reduces yield, plants growing under saline conditions are stressed in several ways: reduction of water potential in the root zone causing water deficit; phytotoxicity of ions caused mainly by Na⁺ and Cl⁻; nutrient imbalance by depression in uptake or/and shoot transport and oxidative damage^{1; 3; 4; 5}.

Common bean is one of the most important legume crops; it contains considerable amounts of protein, fiber, carbohydrates, vitamins and minerals, and represents nearly half of the consumed grain legumes worldwide⁶. The presence of phytochemicals such as polyphenolic compounds prevents various disorders like cardiovascular disease, blood glucose, obesity and colon cancer; in addition they show high antioxidant activity^{7;8}. Common bean is also vital in agriculture as with nitrogen fixing bacteria, it forms root nodules via symbiotic associations⁹. Being a glycophyte species, common bean sees its productivity drastically reduced at soils salinity levels more than 2 dS m⁻¹¹⁰. Thus a better understanding of physiological and biochemical responses under salinity stress can be of value in programs conducted to breed salt tolerant crops.

A diallel cross by definition is the set of all possible matings between several genotypes¹¹. The choice of parents is one of the most crucial aspects, when starting a genetic breeding program¹² therefore according to Griffing¹³ the model I (fixed-effects) is the most appropriate for such studies. Genetic analysis between individuals and populations are important in breeding programs that

involve hybridization or diallel cross mating, because it is easier to identify parents that produce heterotic progenies and results in a higher probability of obtaining superior genotypes in segregating generations¹⁴.

Falconer¹⁵ attested that in genetic breeding programs, genetic dissimilarity is fundamentally important in choosing genotypes to be used as parents; in addition, an inference can be made from diallel crosses computing general combining ability of parents and specific combining ability of hybrids¹³. Such information is helpful for breeders to identify the best combiners which may be hybridized to build up favorable fixable genes¹⁶.

Several research have been carried out to estimate salinity stress effects on common bean, evaluating biochemical, morphological, physiological and phenological traits^{17; 18; 19; 20; 21; 5}. As well as for genetic analysis, including GCA, SCA, heterosis and heritability, where it has been proved that additive and non-additive genes were controlling these traits^{22; 23; 24; 25; 26; 27}, in the contrary Iqbal et al.²⁸ and Ceyhan et al.²⁹ reported that non-additive genes were more involved in some physiological and biochemical traits.

Studies associated both diallel analysis and salinity tolerance have been carried out in many crops e.g. barley¹⁷, tomato³⁰, maize³¹, wheat³² and rice³³, while we couldn't find in the literature research associated both diallel analysis and salinity tolerance for common bean; hence the interest of our research presented in this paper.

In our study, we investigated the nature of gene action influencing biochemical, physiological and phenological traits under saline conditions using diallel cross mating. Combining abilities of the secondary metabolites under study were evaluated, in order to better characterize common bean response to salt stress tolerance and identify limiting factors; useful for developing breeding strategies in order to improve both the productivity and nutritive quality of common bean under salt stress.

1) Materials and methods :

1-1 Plant materials, cross model and growth conditions :

From six parental genotypes of common bean cultivars commonly cultivated in Algeria, four were selected on a preliminary study based on salinity screening experiments (data not shown), and their seed coat color (Table 1).

A one-way diallel cross excluding reciprocals was used to produce six F_1 hybrids during the summer of 2016, the hybrids and their parents were first surface sterilized by immersion in 5% (v/v) commercial Sodium hypochlorite NaClO, then rinsed three times with sterile distilled water and germinated in moist growing medium at 25.5°C for 72 hours, at the Tennessee State University. They were transferred in pots (24x21.8 cm) filled with growing medium (75% peat, vermiculite, perlite, limestone, wetting agent) and grown in a growth chamber under controlled environmental conditions. The conditions maintained during the experiments were: light duration - 14 h, temperature- 27± 2°C and relative air humidity - 65± 5%. A commercial fertilizer (Scotts Miracle-Gro Product, N:P:K (24:8:16), 0.7 kg m⁻³) was used during all the experiments.

1-2 Salt treatments :

The salt treatments: 50 mM NaCl (S1) and 100mM NaCl (S2) was applied gradually to avoid osmotic shock by adding 25mmol/L of NaCl at the appearance of the first trifoliate every four days until to reach the desired concentrations. The control treatment (S0) was irrigated only with water. pH and electrical conductivity (EC) (dS m⁻¹) were measured during the experiment and maintained at the desired levels (pH = 5.5 to 6.5; EC < 2 dS m⁻¹; EC = 5 dS m⁻¹ and EC = 10 dS m⁻¹ for 0, 50 and 100mM NaCl respectively).

1-3 Polyphenolic measurements :

Total phenolic content and total flavonoid content were evaluated as described by Heimler et al.³⁴. For that a 0.5 g sample of ground dry seeds from each genotype was extracted with 45 mL of 70% ethanol and adjusted to pH 2.0 with formic acid; and left for one night at room temperature. The extracts were defatted three times using 15 mL of petroleum ether. The extracts were evaporated to dryness under vacuum at room temperature and then redissolved in EtOH/H₂O (70:30) adjusted to pH 2.0 with formic acid, to a final volume of 1 mL. All data are mean values of three repetitions.

1-3-1 Total phenolic content:

The Folin-Ciocalteu method was used to determine the total phenolic content, described by Heimler et al.³⁴. 0.5 mL of deionized water and 125 μ L of the Folin-Ciocalteu reagent were added to 125 μ L of the diluted sample extract. The mixture was allowed to stand for 6 min, after that 1.25 mL of a 7% aqueous Na₂CO₃ solution was added, and then adjusted to 3 mL as a final volume. The mixture was allowed to stand for 90 min, and the absorption was measured at 760 nm against water as a blank. The amount of total phenolics is expressed as gallic acid equivalents (GAE, mg gallic acid/g sample) through the calibration curve of gallic acid. ($R^2=0.9979$)

1-3-2 Total flavonoid content:

The colorimetric method described by Heimler et al.³⁴ was used to determine the total flavonoid content. 75 μ L of a 5% NaNO₂ solution, 0.150 mL of 10% AlCl₃ solution freshly prepared, and 0.5 mL of 1 M NaOH solution were added to 0.25 mL of the diluted samples. The final volume was adjusted to 2.5 mL with deionized water. The mixture was allowed to stand for 5 min, and the absorption was measured at 510 nm against the same mixture, without the sample as a blank. The amount of total flavonoids is expressed as (+)-catechin equivalents (CE, mg (+)-catechin/g sample) through the calibration curve of (+)-catechin. ($R^2=0.998$).

1-4 Statistical analysis :

Tukey's HSD test was used for comparison between the treatments. The general and specific combining abilities variances and their effects were calculated according to Griffing's method 2 (half diallel crosses, excluding reciprocals) $n(n-1)/2$, model 1(fixed effect). Diallel analysis was carried-out according to Griffing¹³, numerical approach as adopted by Sharma³⁵. ANOVA of the diallel data set showed that the mean squares due to GCA and SCA were significant for all the traits and under all conditions except for DDM under 100 mM NaCl relative to SCA, while the GCA/SCA ratio of mean squares for all studied traits in common bean genotypes was higher than unity (1).

2) Results :

2-1 Diallel analysis and its genetic components:

The statistical analysis revealed high significant differences among the parents and their F_1 hybrids for all the traits (Table 2). In this concern the detailed analysis of general and specific combining ability and the type of gene action was therefore appropriate for estimating the traits investigated during the study. ANOVA of the diallel data set showed that the mean squares due to GCA and SCA were significant for all the traits and under all conditions, while the GCA/SCA ratio of mean squares for all studied traits in common bean genotypes was higher than unity (1).

2-2 Mean performance of parents and their hybrids :

The mean performance of the parents and their six hybrids are presented in Table 3. All the traits under study were significantly affected by salinity. The total phenolic content increases under moderate salt treatment for all the genotypes except the parent P_4 and the hybrids $P_1 \times P_4$ and $P_3 \times P_4$, the parent P_1 registered the highest increase between the parental genotypes, while the hybrids

$P_2 \times P_3$ and $P_2 \times P_4$ recorded the highest TPC production increase and to a lesser extent the hybrids $P_1 \times P_2$ and $P_1 \times P_3$; the cross $P_3 \times P_4$ recorded the highest decrease (1.10). Under 100 mM NaCl the parental genotype P_3 and the crosses $P_1 \times P_2$, $P_1 \times P_3$ and $P_2 \times P_3$ registered a phenolic production increase, the highest increase was recorded by the cross $P_1 \times P_3$; at the same time the hybrid $p3 \times p4$ showed the highest decrease (2.97).

The total flavonoid content decreases under 50 mM NaCl for all the parents and hybrids except the parent P_2 and the hybrids $P_1 \times P_2$; $P_2 \times P_3$ and $P_2 \times P_4$; the maximum decrease and increase were recorded in the hybrids $P_3 \times P_4$ (0.01) and $P_2 \times P_3$ (0.13) respectively, under 100 mM NaCl the total flavonoid content increases for the parental genotype P_2 (MGT) and all the hybrids except for $P_2 \times P_4$ and $P_3 \times P_4$; the maximum increase and decrease was recorded in the hybrids $P_1 \times P_3$ (0.16) and $P_3 \times P_4$ (0.06) respectively.

2-3 Combining ability effects:

The magnitudes of GCA and SCA effects are indicative of the relative importance of additive and non-additive gene actions in the inheritance of a trait, therefore we proceed to the computation of these effects, the estimates of GCA and SCA effects for different genotypes are listed in Table 4 and Table 5, respectively.

For total phenolic content only the parents P_1 (0.70) (p-value < 0.05) and P_4 (1.48) (p-value < 0.01) showed significant positive GCA effects under 100 mM NaCl and normal conditions respectively, on the other hand the cross $P_3 \times P_4$ (1.34) (p-value < 0.05) recorded significant positive SCA effects under non-saline conditions.

For total flavonoid content positive significant GCA effects were recorded for P_1 under normal conditions (0.03) and 100 mM NaCl (0.05) (p-value < 0.01), while the parents P_2 (0.04) (p-value < 0.05) and P_4 (0.05) (p-value < 0.01) showed also positive significant effects under 50 mM NaCl and non-saline conditions respectively, on the other hand according to SCA effects only the cross $P_2 \times P_4$ (0.06) (p-value < 0.05) exhibited significant positive value under 50 mM NaCl.

3) Discussion :

Salt stress is a major environmental factor which prevents plants from attaining their full genetic potential, in the salt sensitive common bean salinity induces several growth limitations². All the parental genotypes and their F_1 hybrids showed more or less susceptibility as a response to salt stress, this response is a complex mixture of phenological, physiological and biochemical genetic expression; the parental genotypes P_1 and P_4 as well as the hybrids $P_1 \times P_2$, $P_1 \times P_3$ and $P_2 \times P_4$, showed relative tolerance to salinity, this tolerance was expressed by a consequent reduction in time for pods maturity, less reduction on yield traits (data not shown) and higher production of antioxidative compounds, and even if yield decreases causing economic losses, with appropriate management practices these losses can be offset by the production of higher quality seed containing considerable amount of antioxidant compounds, that can be commercialized to meet the changing demands of the market and the consumer health; in addition all statistical analysis were highly significant under all conditions, these results demonstrate evidence of the presence of a large genetic variability among the parents and their respective hybrids which may facilitate salt tolerance and genetic improvement using such genetic material of common bean. These results are in agreement with previous results for common bean and other crops^{19; 23; 20; 5; 36}.

The GCA variance contains additive epistasis effect, while SCA variance contains non-additive effects¹³. The analysis of variance for GCA and SCA were highly significant for all the traits under all conditions, these results confirm that both additive and non-additive genes were involved in controlling these characters through all common bean genotypes; on the other hand GCA effects provide appropriate criterion for detecting the validity of genotype in hybrid combination, while SCA effects may be related to heterosis³⁷. Our results revealed that GCA effects for the traits under study were related to several SCA values of their corresponding crosses, i.e. where the two parents P_1 and P_4 were involved, the crosses produced, namely $P_1 \times P_3$ and $P_2 \times P_4$

recorded highly significant positive SCA effects under saline conditions, this may indicate that additive and non-additive genetic systems present in the crosses are acting in the same direction to maximize the traits in view¹⁶, these results confirmed those finding by several researches^{22; 23; 25; 26; 27}, while they partially contradict those reported by Iqbal et al.²⁸ and Ceyhan et al.²⁹ where they both found significant GCA and SCA mean squares with preponderance of SCA component in their genetic studies on common bean. The GCA/SCA ratio was higher than unity (1) under all salinity levels; therefore the contribution of additive gene action in the genetic expression of the traits under study is greater than the non-additive (dominant) gene action, and then the selection could be effective for salinity tolerance through our common bean materials. These results agree with (Islam et al.³⁰; Atnaf et al.²⁴).

And so on

The results of our study showed that there was a considerable genetic difference between the four genotypes for salinity tolerance; this tolerance is genetically expressed by both additive and non-additive gene action controlling the different biochemical traits. Therefore Améliore roza and Coco nain were good general combiners for the polyphenolic production under different salinity treatments. The crosses MGT x Améliore roza and Coco nain x Amina showed good specific combining ability under different salinity level.

In conclusion, the present results revealed that several of the obtained crosses are highly promising to be used in breeding programs of common bean cultivars which possess potentially genetic factors for salinity tolerance; the hybrids MGT x Améliore roza and Coco nain x Amina can increase beans productivity in saline area limiting economic loss and improving the nutritional quality; the gain of the nutritional quality of beans produced in these area could compensate for the economic loss.

Appendix tables and graphs

Table1: Parental genotypes used for the study.

Parents	Salinity resistance	Seed shape and coat phenotype
Coco Nain (P1)	Moderately resistant	Large, white with brown stripes
Amina (P2)	Sensitive	Small, white
MGT (P3)	Moderately sensitive	Medium kidney, Dark red
Améliore Roza (P4)	Moderately resistant	Large, white with red stripes

Table 2: Mean squares obtained from ANOVA and combining abilities for the studied traits.

Characters	Salinity Level	Genotypes	GCA	SCA	GCA/SCA	Error
Total phenolic content						
	S0	10.86**	9.21**	0.83*	11.15	0.21
	S1	11.82**	7.51**	2.16*	3.48	0.73
	S2	7.25**	3.67**	1.79**	2.05	0.23
Total flavonoid content						
	S0	0.02**	0.01**	0.0009**	15.12	0.0002
	S1	0.02**	0.02**	0.0022*	6.84	0.0008
	S2	0.01**	0.01**	0.0014*	7.87	0.0005

*, ** Significant at the 0.05 and 0.01 probability level, respectively. S0: Control, S1: 50 mM NaCl, S2: 100 mM NaCl.

Table 3: Mean performance of parental cultivars and F1 hybrids for biochemical traits.

Genotype							
	Traits	TPC (mg/g)			TFC (mg/g)		
	Salt level	S0	S1	S2	S0	S1	S2
P1		6.05a	6.42a	5.19ab	0.22a	0.20a	0.22a
P2		3.83a	4.83a	3.35ab	0.08a	0.15b	0.10a
P3		1.44a	1.61a	1.49a	0.02a	0.00a	0.00a
P4		6.65a	6.50a	6.54a	0.23a	0.17b	0.21a
P1xP2		3.81a	4.48a	5.99b	0.15a	0.21b	0.18b
P1xP3		2.26a	2.86a	5.92b	0.08a	0.07a	0.16b
P1xP4		5.72a	4.43b	4.42b	0.15a	0.13a	0.16a
P2xP3		2.05a	4.56b	4.31b	0.05a	0.13b	0.12b
P2xP4		5.79a	6.80a	4.88ab	0.18a	0.24b	0.15c
P3xP4		5.68a	1.10b	2.97c	0.12a	0.01b	0.06c
mean		4.32	4.36	4.50	0.12	0.13	0.13
Tukey's HSD test			1.13			0.038	

S0: control; S1: 50 mM NaCl; S2: 100 mM NaCl. TPC : Total phenolic content; TFC: Total flavonoid content.
 Values in the same row followed by the same letters do not differ significantly (Tukey's HSD test, $P < 0.05$)

Table 4: Estimates of genotype general combining ability effects (GCA) for biochemical traits and its components.

Trait	Total phenolic content			Total flavonoid content		
	S0	S1	S2	S0	S1	S2
P1	0.37	0.47	0.70*	0.03**	0.03	0.05**
P2	-0.39	0.62	-0.11	-0.02*	0.04*	-0.01
P3	-1.46**	-1.68*	-1.06**	-0.06**	-0.07**	-0.06**
P4	1.48**	0.59	0.47	0.05**	0.01	0.02
S.E gi	0.16	0.30	0.17	0.005	0.01	0.01
S.E gi-gj	0.26	0.49	0.28	0.01	0.02	0.01

*, ** Significant at the 0.05 and 0.01 probability level, respectively. S0: control; S1: 50 mM NaCl; S2: 100 mM NaCl. S.E: Standard error.

Table 5: Estimates of genotype specific combining ability effects (SCA) for biochemical traits and its components.

Trait	Total phenolic content			Total flavonoid content		
	S0	S1	S2	S0	S1	S2
P1xP2	-0.51	-0.96	0.89	0.004	0.02	0.005
P1xP3	-0.98*	-0.29	1.77**	-0.02	-0.01	0.04
P1xP4	-0.46	-0.98	-1.26*	-0.05**	-0.04	-0.04
P2xP3	-0.42	1.26	0.97	-0.002	0.03	0.04
P2xP4	0.38	1.23	0.01	0.03	0.06*	-0.001
P3xP4	1.34*	-2.17*	-0.94	0.005	-0.06	-0.04
S.E sij	0.39	0.73	0.41	0.01	0.02	0.02
S.E sij-sik	0.59	1.10	0.62	0.02	0.04	0.03

*, ** Significant at the 0.05 and 0.01 probability level, respectively. S0: control; S1: 50 mM NaCl; S2: 100 mM NaCl. S.E: Standard error.

Referrals and references:

- ¹ Khan, M.H., Panda, S.K., 2008. Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. *Acta Physiol. Plant.* 30, 81–89.
<https://doi.org/10.1007/s11738-007-0093-7>
- ² Gama, P.B.S., Inanaga, S., Tanaka, K., Nakazawa, R., 2007. Physiological response of common bean (*Phaseolus vulgaris* L.) seedlings to salinity stress. *J. Biotechnol.* 6, 79–88.

- ³ Rozena, J., Flowers, T., 2008. Crops for a salinized world. *Science*, 322, 1478.
- ⁴ James, R.A., Blake, C., Byrt, C.S., Munns, R., 2011. Major genes for Na⁺ exclusion, Nax1 and Nax2 (wheat HKT1;4 and HKT1;5), decrease Na⁺ accumulation in bread wheat leaves under saline and waterlogged conditions. *J. Exp. Bot.* 62, 2939–2947. <https://doi.org/10.1093/jxb/err003>
- ⁵ Taïbi, K., Taïbi, F., Ait Abderrahim, L., Ennajah, A., Belkhodja, M., Mulet, J.M., 2016. Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in *Phaseolus vulgaris* L. *South African J. Bot.* 105, 306–312. <https://doi.org/10.1016/j.sajb.2016.03.011>
- ⁶ Duc, G., Agrama, H., Bao, S., Berger, J., Bourion, V., De Ron, A.M., Gowda, C.L.L., Mikic, A., Millot, D., Singh, K.B., Tullu, A., Vandenberg, A., Vaz Patto, M.C., Warkentin, T.D., Zong, X., 2015. Breeding Annual Grain Legumes for Sustainable Agriculture: New Methods to Approach Complex Traits and Target New Cultivar Ideotypes. *CRC. Crit. Rev. Plant Sci.* 34, 381–411. <https://doi.org/10.1080/07352689.2014.898469>
- ⁷ Pinheiro, C., Baeta, J.P., Pereira, A.M., Domingues, H., Ricardo, C.P., 2010. Diversity of seed mineral composition of *Phaseolus vulgaris* L. germplasm. *J. Food Compos. Anal.* 23, 319–325. <https://doi.org/10.1016/j.jfca.2010.01.005>
- ⁸ Hayat, I., Ahmad, A., Masud, T., Ahmed, A., Bashir, S., 2014. Nutritional and Health Perspectives of Beans (*Phaseolus vulgaris* L.): An Overview. *Crit. Rev. Food Sci. Nutr.* 54, 580–592. <https://doi.org/10.1080/10408398.2011.596639>
- ⁹ Broughton, W.J., Hernandez, G., Blair, M., Beebe, S., Gepts, P., Vanderleyden, J., 2003. Beans (*Phaseolus* spp.): model food legumes. *Plant Soil* 252, 55–128. <https://doi.org/10.1023/A:1024146710611>
- ¹⁰ Läuchli, A., 1984. Salt exclusion: an adaptation of legumes for crops and pastures under saline conditions. R.C. Staples, G.H. Toenniessen (Eds.), *Salinity tolerance in plants: Strategies for crop improvement*, Wiley, New York, pp. 171–187
- ¹¹ Hayman, B.I., 1954. The theory and analysis of diallel crosses. Unit of Biometrical Genetics, Department of Genetics, University of Birmingham.
- ¹² Correa, A.M., De Souza Lima, A.R., Braga, D.C., Ceccon, G., Teodoro, P.E., Da Silva Junior, C.A., Da Silva, F.A., 2015. Agronomic performance and genetic variability among common bean genotypes in savanna/pantanal ecotone. *J. Agron.* 14, 175–179. <https://doi.org/10.3923/ja.2015.175.179>
- ¹³ Griffing, B., 1956. Concept of General and Specific Combining Ability in Relation to Diallel Crossing Systems. *Aust. J. Biol. Sci.* 9, 463. <https://doi.org/10.1071/BI9560463>
- ¹⁴ Benitez L.C., da Silva Rodrigues I.C., Arge L.W.P., Ribeiro M.V., Braga E.J.B., 2011. Multivariate analysis of genetic divergence of genotypes of rice under salt stress during the vegetative phase. *Rev. Cienc. Agron.* 42: 409–416. <https://doi.org/10.1590/S1806-66902011000200021>

- ¹⁵ Falconer, D.S., 1981. Introduction to quantitative genetics. 2nd edn. Longman, London.
- ¹⁶ Ghareeb Zeinab, E., Helal, A.G., 2014. Diallel analysis and separation of genetic variance components in eight faba bean genotypes. Ann. Agric. Sci. 59, 147–154. <https://doi.org/10.1016/j.aoas.2014.06.019>
- ¹⁷ Chen, Z., Shabala, S., Mendham, N., Newman, I., Zhang, G., Zhou, M., 2008. Combining ability of salinity tolerance on the basis of NaCl- induced KC flux from roots of barley. Crop Sci. 48, 1382–1388. doi: 10.2135/cropsci2007.10.0557
- ¹⁸ Zuccarini, P., 2008. Effects of silicon on photosynthesis, water relations and nutrient uptake of *Phaseolus vulgaris* L. under NaCl stress. Biol. Plant. 52, 157–160.
- ¹⁹ Cabot, C., Sibole, J. V., Barceló, J., Poschenrieder, C., 2009. Absciscic acid decreases leaf Na⁺ exclusion in salt-treated *Phaseolus vulgaris* L. J. Plant Growth Regul. 28, 187–192. <https://doi.org/10.1007/s00344-009-9088-5>
- ²⁰ Talaat, N.B., 2015. Effective Microorganisms Improve Growth Performance and Modulate the ROS-Scavenging System in Common Bean (*Phaseolus vulgaris* L.) Plants Exposed to Salinity Stress. J. Plant Growth Regul. 34, 35–46. <https://doi.org/10.1007/s00344-014-9440-2>
- ²¹ Bargaz, A., Nassar, R.M.A., Rady, M.M., Gaballah, M.S., Thompson, S.M., Brestic, M., Schmidhalter, U., Abdelhamid, M.T., 2016. Improved Salinity Tolerance by Phosphorus Fertilizer in Two *Phaseolus vulgaris* Recombinant Inbred Lines Contrasting in Their P-Efficiency. J. Agron. Crop Sci. 202, 497–507. <https://doi.org/10.1111/jac.12181>
- ²² Arunga, E.E., Rheenen, H.A. Van, Owuoche, J.O., 2010. Diallel analysis of Snap bean (*Phaseolus vulgaris* L.) varieties for important traits. African J. Agric. Res. 5, 1951–1957. <https://doi.org/10.5897/AJAR09.082>
- ²³ Trindade, R.S., Rodrigues, R., Amaral Júnior, A.T., Gonçalves, L.S.A., Viana, J.M.S., Sudré, C.P., 2015. Combining ability for common bacterial blight resistance in snap and dry bean (*Phaseolus vulgaris* L.). Acta Scientiarum. Agronomy 37: 37-43.
- ²⁴ Atnaf, M., 2013. Inheritance of primary yield component traits of common beans (*Phaseolus vulgaris* L.): Number of seeds per pod and 1000 seed weight in an 8 x 8 diallel cross population. Int. J. Genet. Mol. Biol. 5, 42–53. <https://doi.org/10.5897/IJGMB2013.0076>
- ²⁵ Bi, Y., Li, W., Xiao, J., Lin, H., Liu, M., Liu, M., Luan, X., Zhang, B., Xie, X., Guo, D., Lai, Y., 2015. Heterosis and combining ability estimates in isoflavone content using different parental soybean accessions: Wild soybean, a valuable germplasm for soybean breeding. PLoS One 10, 1–13. <https://doi.org/10.1371/journal.pone.0114827>
- ²⁶ Senbetay, T., Varieties, V.L., 2015. Combining Ability of Commercial White Pea Bean (*Phaseolus vulgaris* L.) Varieties in South Western Ethiopia. J. Biol. Agric. Healthc. 5, 153–162.
- ²⁷ da Silva, F.A., Corrêa, A.M., Teodoro, P.E., Lopes, K.V., Corrêa, C.C.G., 2017. Genetic divergence in the common bean (*Phaseolus vulgaris* L.) in the Cerrado-Pantanal ecotone. Genet. Mol. Res. 16, 1–11. <https://doi.org/10.4238/gmr16019570>

- ²⁸ Iqbal, A.M., Nehvi, F.A., Wani, S.A., Dar, Z.A., Lone, A.A., Qadri, H., 2012. COMBINING ABILITY STUDY OVER ENVIRONMENTS IN DRY BEANS (*Phaseolus vulgaris* L.). SAARC J. Agri. 10, 61–69.
- ²⁹ Ceyhan, E., Harmankaya, M., Kahraman, A., 2014. Combining ability and heterosis for concentration of mineral elements and protein in common bean (*Phaseolus vulgaris* L.). Turkish J. Agric. For. 38, 581–590. <https://doi.org/10.3906/tar-1307-56>
- ³⁰ Pandey, S.K., Mall, A.K., 2015. Analysis of Heterosis on Morphological and Qualitative Traits in Tomato (*Solanum Lycopersicon* Mill) for Salinity Tolerance . Int. J. Agric. Environ. Biotechnol. 8, 423–431.
- ³¹ Islam, M., Sohail, Q., Maqbool, M.A., Zaman, Q.U., Ahmed, Z., 2015. Combining ability analysis and genetic inheritance of salt tolerance indicators in maize (*Zea mays*) following diallel mating design. Int. J. Agric. Biol. 17, 523–530. <https://doi.org/10.17957/IJAB/17.3.14.472>
- ³² Baloch, M.J., Channa, G.M., Jatoti, W.A., Baloch, A.W., Rind, I.H., Arain, M.A., Keerio, A.A., 2016. Genetic Characterization in 5 × 5 Diallel Crosses for Yield Traits in Bread Wheat. Sarhad J. Agric. 32, 127–133. <https://doi.org/10.17582/journal.sja/2016/32.3.127.133>
- ³³ Mohammadi, R., Mendioro, M.S., Diaz, G.Q., Gregorio, G.B., Singh, R.K., 2014. Genetic analysis of salt tolerance at seedling and reproductive stages in rice (*Oryza sativa*). Plant Breed. 133, 548–559. <https://doi.org/10.1111/pbr.12210>
- ³⁴ Heimler, D., Vignolini, P., Dini, M.G., Romani, A., 2005. Rapid tests to assess the antioxidant activity of *Phaseolus vulgaris* L. dry beans. J. Agric. Food Chem. 53, 3053–3056. <https://doi.org/10.1021/jf049001r>
- ³⁵ Sharma Jawahar R., 2006. Statistical and Biometrical Techniques in Plant Breeding. New Age International.
- ³⁶ Agbahoungba, S., Karungi, J., Badji, A., Sadik, K., Gibson, P., Edema, R., Assogbadjo, A.E., Rubaihayo, P.R., 2018. Inheritance of cowpea resistance to flower thrips in Uganda germplasm. J. Plant Breed. Crop Sci. 10, 21–32. <https://doi.org/10.5897/JPBCS2017.0698>
- ³⁷ Harriman, J.C., Nwammadu, C.A., 2016. Utilization of diallel analyses for heritability, GCA and SCA studies in crop improvement. Am. Adv. J. Biol. Sci. 1, 159–167. <https://doi.org/10.18869/AAJBS.2016.159>