Genetic divergence of physiological-quality traits of seeds in a population of peppers

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ABSTRACT. Brazil has a great diversity of *Capsicum* peppers that can be used in breeding programs. The objective of this study was to evaluate genetic variation in traits related to the physiological quality of seeds of *Capsicum annuum* L. in a segregating F_2 population and its parents. A total of 250 seeds produced by selfing in the F_1 generation resulting from crosses between UFPB 77.3 and UFPB 76 were used, with 100 seeds of both parents used as additional controls, totaling 252 genotypes. The seeds were germinated in gerboxes containing substrate blotting paper moistened with distilled water. Germination and the following vigor tests were evaluated: first count, germination velocity index, and root and shoot lengths. Data were subjected to analysis of variance, and means were compared by Scott and Knott's method at 1% probability. Tocher's clustering based on Mahalanobis distance and canonical variable analysis with graphic dispersion of genotypes were performed, and genetic parameters were estimated. All variables were found to be significant by the F test ($P \leq 0.01$) and showed high heritability and a CVg/CVe ratio higher than 1.0, indicating genetic differences among genotypes. Parents (genotypes 1 and 2) formed distinct groups in all clustering methods. Genotypes 3, 104, 153, and 232 were found to be the most divergent according to Tocher's

clustering method, and this was mainly due to early germination, which was observed on day 14, and would therefore be selected. Understanding the phenotypic variability among these 252 genotypes will serve as a basis for continuing the breeding program within this family.

Key words: Breeding; Genetic Resources; Germination; Heritability; Seed vigor

INTRODUCTION

In the last few years, the pepper (*Capsicum*) agri-business has gained increasing ground in the market, and is now considered one of the best examples of integration between those involved in its production chain, especially among family farmers, who are mainly responsible for the growth of this activity (Ohara and Pinto, 2012). Peppers are successfully marketed based ontheir numerous forms of commercial use, their high added value, and their medicinal and, more recently, ornamental uses (Rêgo et al., 2012a,b; Ohara and Pinto, 2012). The species *Capsicum annuum* shows large variability that can be of ornamental interest given the great diversity observed in many traits such as size, foliage, and color of the vegetables (Finger et al., 2012; Silva Neto et al., 2014).

Peppers usually propagate through their seeds, which should have a high physiological potential that includes rapid and uniform germination, plantlets with greater tolerance to environmental adversities, and a uniform maturity of the crop, which results in increased productivity and a shorter time to obtain the seedlings (Bennett, 2001). Therefore, the need for studies and techniques that contribute to potentiating the germination process in peppers is justified, since, according to Fialho et al. (2010), their rates of germination and plantlet-emergence are low.

In this scenario, identifying superior genotypes in terms of the physiological quality of seeds can help to obtain more vigorous and earlier varieties, and should be a factor in the study of breeding programs. Genetic factors that can affect the quality of seeds are related to differences in vigor and longevity observed within the same species, as well asthe advantages provided by heterosis (Cardoso et al., 2009). Hence, the study of genetic divergence and genetic relationships is of paramount importance for the selection of the traits related to the physiological quality of seeds.

Therefore, the objective of this study was to evaluate genetic variation based on the physiological quality of seeds of *C. annuum* L. in a segregating F_2 population and in its parents, and to determine the traits that contribute most to the observed divergence.

MATERIAL AND METHODS

The experiment was conducted in the Laboratory of Plant Biotechnology of Universidade Federal da Paraíba (UFPB), Center for Agricultural Sciences (CCA), located in Areia, PB, Brazil. Two parents of *C. annuum* belonging to the Vegetable Germplasm Bank of CCA-UFPB were used: accession No. UFPB 77.3 and UFPB 76, which were crossed to obtain the F_1 generation; these plants were later self-pollinated to obtain the F_2 generation. One hundred seeds from each parent and 250 seeds from the F_2 generation were used to evaluate the physiological quality of the seeds. Percentage of germination (at 14 and 21 days); germination velocity index (GVI); root length (RL); and shoot length (SL) were evaluated as recommended by Rules for Seeds Analyses (RAS) (Brasil, 2009).

The experiment was performed in a completely randomized block design with additional controls (Cruz, 2006). Two parents, with four replicates of 50 seeds, were employed as controls. The F_2 family comprised 250 seeds. Data were transformed according to the method described by Bartlett (1947). After transformation, the data were subjected to analysis of variance, and means were clustered by Scott-Knott's criterion at 1% probability. The estimates of heritability in the broad sense, genetic variance, and the ratio between the coefficients of genetic and environmental variation were also calculated.

Tocher's method (Rao, 1952) based on generalized Mahalanobis distance and analysis of canonical variables with graphic dispersion of the genotypes was used to analyze genetic divergence. The relative importance of the variables was determined by the method described by Singh (1981) and by canonical variables. All analyses were performed on the GENES computer software (Cruz, 2006).

RESULTS

Significant differences were observed in the analysis of variance by the F test ($P \leq 0.01$) for the five traits related to the physiological quality of the seeds, demonstrating the existence of genetic variability among the 252 studied genotypes of *C. annuum* (Table 1). The observed heritability values were above 95%, with the exception of the trait percentage of germination, which showed 89.78% heritability, and was still considered to be high.

The ratio between the coefficient of genetic variation and the coefficient of environmental variation (CVg/CVe) was greater than 1.0 for the variables first germination count, GVI, RL, and SL. For the percentage of germination, the CVg/CVe ratio was 0.2965.

The coefficients of variation (CV%) of the experiment varied from 2.272% for the germination speed index, to 69.266% for root length, which are thus satisfactory, since significant differences were detected among genotypes.

According to Scott-Knott's test at 1% probability, the genotypes were classified into only two groups for all evaluated traits (Table 1). For the variable first germination count, only one of the parents had germinated (accession No. 77.3), and were thus found to be earlier and more vigorous than the second parent (GenBank accession No. 76), which did not germinate within the experimental period. In the F_2 generation, only the seeds from genotypes 3, 5, 7, 103, 104, 105, 106, 107, 108, 115, 153, and 203 produced normal plantlets.

On day 21, most of the genotypes already showed germination (Table 1). Genotype 3 had the highest GVI (0.7906 [0.125]), which did not differ from that of the genotypes that germinated. On the other hand, the genotypes that did not germinate in the experimental period were integrated into the second group in which accession 76 is located (Parent 1). The genotypes were clustered into two groups for variables RL and SL: 127 genotypes formed group one, with parent 77.3; and 123 genotypes formed group two, with parent 76.

The adoption of Tocher's optimization method based on Mahalanobis distance allowed 252 individuals to be clustered into 12 groups (Table 2). Groups I and II comprised the majority of the *C. annuum* genotypes, 121 (48.01%) and 95 (37.69%), respectively. Groups III, IV, and VI were each formed by six genotypes (2.38%); whereas group V consisted of five genotypes (1.98%); groups VII and VIII were composed of four genotypes each (1.58%); and groups IX, X, XI, and XII, were formed by only one genotype each: 3, 104, 153 and 232, respectively.

Table 1. Summary of the analysis of variance: mean squares (MS), heritability (h^2), ratio between the coefficients of genetic and environmental variation (CVg/CVe) and coefficient of variation (CV), number of groups, and junction of genotypes per group for five variables related to the physiological quality of seeds in parents and segregating generation of ornamental peppers (*Capsicum annuum* L.). CCA/UFPB, 2013.

SV	MS				
	Germination 14th day	Germination 21st day	GVI	RL	SL
Treatment	4.0154*	0.0521*	0.5206*	292.500*	173.774*
h^2 (%)	99.73	89.78	99.93	99.293	99.37
CVg/CVe	1.927	0.2965	3.894	1.185	1.259
CV (%)	10.303	8.270	2.272	69.266	50.02
N of groups	2	2	2	2	2
Groups of genotypes	1) P77.3 03 05 07 103 104 105 106 107 108 115 153 203	1) 06 08 09 10 11 12 13 14 15 16 17 18 19 20 22 23 24 25 26 27 28 30 33 53 55 56 57 58 59 61 63 64 66 67 68 72 73 76 109 110 111 112 113 114 116 117 118 119 120 121 122 123 124 125 126 127 128 129 131 132 133 154 155 156 157 158 159 161 162 163 164 165 166 167 168 169 171 172 174 175 176 179 180 182 183 184 185 186 187 205 206 207 208 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 239 245 246 248 249	1) 02 21 29 31 32 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 54 60 62 65 69 70 71 74 75 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 160 166 177 178 186 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 204 209 215 219 220 222 224 227 228 229 230 231 232 233 234 235 236 237 238 240 241 242 243 244 247 250 251 252	1) 01 03 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 22 23 24 25 26 27 28 30 33 53 55 56 57 58 59 61 63 64 66 67 68 72 73 76 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 131 132 133 153 154 155 156 157 158 159 161 162 163 164 165 167 168 169 171 172 174 175 176 179 180 182 183 184 185 186 187 203 205 206 207 208 210 211 212 213 214 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 232 233 234 239	1) 01 03 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 22 23 24 25 26 27 28 30 33 53 55 56 57 58 59 61 63 64 66 67 68 72 73 76 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 131 132 133 153 154 155 156 157 158 159 161 162 163 164 165 167 168 169 171 172 174 175 176 179 180 182 183 184 185 186 187 203 205 206 207 208 210 211 212 213 214 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 232 233 234 239

Continued on next page

Table 1. Continued.

SV	MS				
	Germination 14th day	Germination 21st day	GVI	RL	SL
Groups of genotypes	2) P76 04 06 08 09 10 11 12 13 14 15	2) 01 02 03 04 05 07 21 29 31	2) 01 03 04 05 06 07 08 09	2) 02 04 21 29 31 32	2) 02 04 21 29 31 32 34
	16 17 18 19 20 21 22 23 24 25 26 27 28	32 34 35 36 37 38 39 40 41 42	10 11 12 13 14 15 16 17	34 35 36 37 38 39 40	35 36 37 38 39 40 41 42
	29 30 31 32 33 24 25 36 37 38 39 40 41	43 44 45 46 47 48 49 50 51 52	18 19 20 22 23 24 25 26	41 42 43 44 45 46 47	43 44 45 46 47 48 49 50
	42 43 44 45 46 47 48 49 50 51 52 53 54	54 60 62 65 69 70 71 74 75 77	27 28 30 33 53 55 56 57	48 49 50 51 52 54 60	51 52 54 60 62 65 69 70
	55 56 57 58 59 60 61 62 63 64 65 66 67	78 79 80 81 82 83 84 85 86 87	58 59 61 63 64 66 67 68	62 65 69 70 71 74 75	71 74 75 77 78 79 80 81
	68 69 70 71 72 73 74 75 76 77 78 79 80	88 89 90 91 92 93 94 95 96 97	72 73 76 103 104 105 106	77 78 79 80 81 82 83	82 83 84 85 86 87 88 89
	81 82 83 84 85 86 87 88 89 90 91 92 93	98 99 100 101 102 103 104	107 108 109 110 111 112	84 85 86 87 88 89 90	90 91 92 93 94 95 96 97
	94 95 96 97 98 99 100 101 102 109 110	105 106 107 108 130 134 135	113 114 115 116 117 118	91 92 93 94 95 96 97	98 99 100 101 102 130
	111 112 113 114 116 117 118 119 120	136 137 138 139 140 141 142	119 120 121 122 123 124	98 99 100 101 102 130	134 135 136 137 138
	121 122 123 124 125 126 127 128 129	143 144 145 146 147 148 149	125 126 127 128 129 130	134 135 136 137 138	139 140 141 142 143
	130 131 132 133 134 135 136 137 138	150 151 152 153 160 166 170	131 132 133 153 154 155	139 140 141 142 143	144 145 146 147 148
	139 140 141 142 143 144 145 146 147	173 177 178 181 188 189 190	156 157 158 159 161 162	144 145 146 147 148	149 150 151 152 160
	148 149 150 151 152 154 155 156 157	191 192 193 194 195 196 197	163 164 165 167 168 169	149 150 151 152 160	166 170 173 177 178
	158 159 160 161 162 163 164 165 166	198 199 200 201 202 203 204	170 171 172 173 174 175	166 170 173 177 178	181 188 189 190 191
	167 168 169 170 171 172 173 174 175	209 215 227 228 229 230 231	176 179 180 181 182 183	181 188 189 190 191	192 193 194 195 196
	176 177 178 179 180 181 182 183 184	232 233 234 235 236 237 238	184 185 187 203 205 206	192 193 194 195 196	197 198 199 200 201
	185 186 187 188 189 190 191 192 193	240 241 242 243 244 247 250	207 208 210 211 212 213	197 198 199 200 201	202 204 209 231 235
	194 195 196 197 198 199 200 201 202	251 252	214 216 217 218 221 223	202 204 209 231 235	236 237 238 240 241
	204 205 206 207 208 209 210 211 212		225 226 239 245 246 248	236 237 238 240 241	242 243 244 245 246
	213 214 215 216 217 218 219 220 221		249	242 243 244 245 246	247 248 249 250 251
	222 223 225 226 227 228 229 230 231			247 248 249 250 251	252
	232 233 234 235 236 237 238 239 240			252	
	241 242 243 244 245 47 248 249 250				
	251 252				

*Significant at 5% probability, by the F test.

Table 2. Clustering of 252 genotypes of *Capsicum annuum* based on variables related to the physiological quality of seeds according to Tocher's method.

Group	Individuals
1	2* 421 29 31 32 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 54 60 62 65 69 70 71 74 75 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 130 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 160 166 170 173 177 178 181 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 204 209 215 231 235 236 237 238 240 241 242 243 244 247 250 251 252 233 234
2	245 249 248 246 187 226 72 179 176 184 76 174 171 27 207 183 133 28 66 64 109 210 20 214 55 56 61 112 205 208 217 239 206 58 223 113 118 53 18 213 218 67 14 127 163 13 221 159 123 25 128 155 73 180 33 111 156 211 9 126 172 185 11 122 57 119 117 116 17 129 26 131 154 30 124 162 225 132 175 23 24 68 169 114 168 165 157 158 125 121 19 216 120 59 161 110
3	7 203 115 5 108 1*
4	152 12 16 164 182 167
5	219 222 224 220 186
6	6 10 22 12 63 8
7	227 228 229 230
8	103 107 106 105
9	104
10	153
11	3
12	232

CCA/UFPB, 2013.

Of the variables studied, those that most contributed to the genetic divergence among the individuals were GVI (61.88%) and germination on day 21 (27.70%), while the remaining three variables contributed to only 10.42% (Table 3); these were shoot length (0.90%), root length (4.42%), and first germination count (5.10%).

Table 3. Estimates of the relative contribution of each variable (S.j) for the genetic divergence among individuals of *Capsicum annuum* L. based on the total participation of D2, for five variables related to the physiological quality of seeds in parents and segregating generation of ornamental peppers.

Variable	Relative contribution	
	S.j	Value (%)
Percentage of germination	1235040.359	27.70
First count of germination	227499.4480	5.10
Germination velocity index	2758414.933	61.88
Root length	197167.0986	4.42
Shoot length	40200.0746	0.90

CCA/UFPB, 2013.

The canonical variables displayed these same variables to be discarded, which was consistent with Singh's method. Estimates of variance associated with the relative canonical variables indicate that the first and second canonical variables represent, respectively, 94.932 and 2.461% of the total variability (Table 4). The first two variables explained over 80% of the total variation.

The graphic dispersion allowed for the separation of 252 genotypes into seven groups, and can be used as a strategy to select genotypes from different groups, assuming that these are more divergent; these can then be used to launch new lines in the F3 generation (Figure 1).

Table 4. Estimate of variance (eigen values) for canonical variables and relative importance (eigen vectors) of five traits related to the physiological quality of seeds in a segregating population of ornamental peppers (*Capsicum annuum* L.).

Canonical variable	EV	EV (%)	Accumulated %	FG	G21	GVI	RL	SL
CV1	66.913	94.932	94.932	-1.836	-1.055	1.637	-0.238	0.049
CV2	1.734	2.461	97.393	-0.407	0.764	0.501	-0.505	0.598
CV3	1.230	1.745	99.139	0.147	-0.199	-0.108	-2.164	2.248
CV4	0.486	0.689	99.829	-0.604	-0.430	0.114	0.780	0.319
CV5	0.120	0.170	100.000	0.941	0.494	0.335	0.078	-0.201

FG = first germination count on the 14th day; G21 = germination count on the 21st day; GVI = germination velocity index; RL = root length (mm); SL = shoot length (mm). CCA/UFPB, 2013.

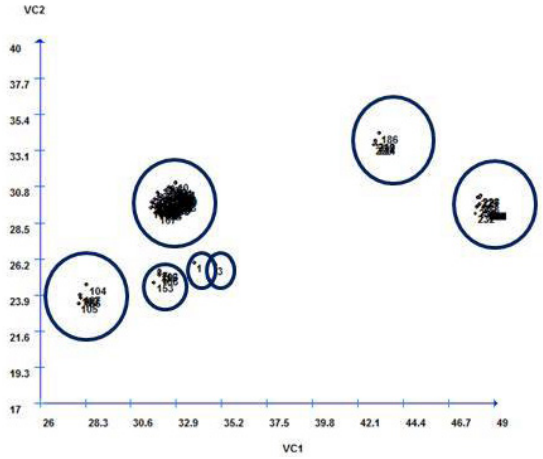

Figure 1. Graphic dispersion of scores in relation to axes representing the canonical variables for five traits related to the physiological quality of seeds in parents and segregating populations of ornamental peppers (*Capsicum annuum* L.). CCA/UFPB, 2013.

Genotypes 3, 104, 153, and 232 should be retained for morpho-agronomic characterization, and could be utilized to launch new lines in the F3 generation.

DISCUSSION

High heritability values found in this study indicate that most of the total variability observed among the genotypes is due to genetic differences (Vencovsky and Barriga, 1992).

During research on papaya, Cardoso et al. (2009) found high heritability values (above 80%) for the traits germination and root length. Germination in *Senna multijuga* also showed heritability above 80% in different populations (Maluf, 1993). However, in progenies of half-siblings of carrot (Daucuscarota), Vieira et al. (2005) observed heritability of 92.82% for germination and 89.23% for vigor, obtained from the variable first count, on day 7.

To our knowledge, no other studies have been performed in *Capsicum* with the aim of estimating heritability of the traits germination and seed vigor. Based on the results of the present study, these traits can be utilized to select genotypes in early generations because of the high heritability of the evaluated traits.

The ratio from CVg/CVe greater than 1 indicates that this condition is desirable in the selection process, as it indicates that the genetic variation has overcome the environmental variation (Cruz et al., 2012; Nascimento et al., 2012). High coefficients of genetic variation and heritability are the main requisites for genetic gain from selection (Falconer and Mackay, 1996).

Variable with low value CVg/CVe indicates that this situation was not very favorable for selection (Vencovsky, 1978). In such cases, new studies of association among traits should be conducted aiming to observe higher genetic correlations, thereby allowing for indirect selection (Cruz et al., 2012) or the practice of selection in advanced generations to occur.

For CV%, in a study on peppers, Silva et al. (2011) found that the CV values usually vary with factors such as trait, accession, and species.

The seeds that did not germinate in the trial period, this is because one of the attributes affecting germination and seed quality is dependent on the genotype, besides other physical, physiological, and sanitary factors (Popinigis, 1977). Belletti and Quagliotti (1989) reported that the percentage of pepper seeds that do not germinate until 14 days after seeding is high, and thus require a period of up to 45 days for most of the seeds from a batch to germinate satisfactorily.

This was also reported by Barbosa (2012), who, in an experiment with *Capsicum chinense*, observed a low percentage of germination, varying from 0 to 53%, in the treatments employed in the first count, which required 21 days for germination.

The genotypes that produced normal plantlets can be indicated for selection of earlier and vigorous genotypes, because the first count of the germination test, performed on day 14, can be used as a vigor test. This is because more vigorous genotypes have a higher germination speed. Thus, genotypes that show germination in the first count can be considered more vigorous (Barbosa, 2012).

Studies conducted by Bhering et al. (2006) with six batches of pepper (*Capsicum frutescens*) seeds demonstrated that there were significant differences between the batches, which were classified into different levels of physiological quality. The authors detected unevenness in germination and vigor (first count), with observed values ranging from 18.5 to 85%; this response was similar to that found in the present study.

To GVI, Barbosa (2012) found values much higher than those found here, varying from 1.9 to 3.32. These differences were also observed for vigor variables RL and SL between parents and the F_2 generation. According to Dan et al. (1987), this occurs because more vigorous seeds generate plantlets with a higher growth rate, because of a greater translocation of reserves from storage tissues to enable growth of the embryonic axis. These differences may be related to genetics, because different genotypes may carry genes that are responsible for an increase or reduction in seed vigor (Casali and Couto, 1984).

In the adoption of Tocher's optimization method based on Mahalanobis, the results show that there is genetic proximity between these individuals, because, according to the methodology of Tocher (Rao, 1952), individuals belonging to the same group are more homogenous than those from distinct groups. Genotypes belonging to different groups should be retained for evaluation of morpho-agronomic traits of interest such as plant height, canopy width, number of fruits, and color of fruits and foliage, which are important for ornamental purposes.

By the method of canonical variables, the last variables will be discarded for future studies within this family. Based on the method described by Singh (1981), Cardoso et al. (2009) evaluated the relative importance of eight variables related to the physiological quality of papaya seeds and highlighted the variables that most contributed to divergence: thousand-seed weight (37.07%), RL (19.00%), and fresh mass of plantlets (17.76%); the variables first count, RL, and SL were

discarded. Variables with a lower contribution were also detected: germination in a greenhouse (1.48%) and GVI (1.54%). These results differ from those found in the present study, emphasizing the importance of this type of study to examine seed characteristics in several species.

When the first two variables explains over 80% of the total variation, indicates that their use is satisfactory in the study of genetic divergence by evaluating the graphic dispersion of the scores (Rêgo et al., 2003; Cardoso et al., 2009; Cruz et al., 2012). This result differed from that observed by Cardoso et al. (2009) and Hernández-Verdugo et al. (2001), in which three canonical variables were required to explain approximately 80% of the total variation based on characteristics of *Carica papaya* and *C. annuum* seeds, respectively.

Through graphic dispersion, we noted that the number of groups differed when Tocher's method of clustering analysis was utilized, as it provided greater separation of the genotypes. Both methods were in agreement for genotype 3, as its spatial positioning in Figure 1 is consistent with the arrangement of the genotype in a group isolated by Tocher's method; therefore, this genotype should be selected for launching a new line in the F3 generation as it shows greater germination and vigor in the provision of earlier plants.

Conflicts of interest

The authors declare no conflict of interest.

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