

Adaptability and stability parameters of total seed yield and protein content in cowpea (*Vigna unguiculata*) genotypes subjected to semi-arid conditions

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Abstract

The selection of superior cultivars through the combination of high yield and protein content cowpea seeds in semi-arid tropical regions have a huge positive impact on the production-market chains in places where there is short availability of protein sources for human consumption. The main aim of the present study is to estimate the adaptability and stability parameters of total seed yield and protein content in cowpea genotypes in order to release new cultivars by combining these value-added traits. Forty-four inbred lines and cultivars were assessed under seven different environmental conditions, either rain-fed or irrigated crop management, in seven sites the Brazilian semi-arid region. The seed protein content was quantified through the Kjeldahl method. Grain yield was adjusted in mean of plant per plot by the covariance method. Statistically significant differences in the genotype as well as in the genotype×environment interaction were observed in all the assays. The inbred lines presenting the highest protein contents showed the lowest grain yields, and it indicated the prominent “phenotypic cost” of protein in overall cowpea seed production. However, the breakage between the herein assessed association was observed in inbred lines subgroups such as ‘C3Q’, ‘C3M’, ‘C2S’, and ‘CIJ’. These lines showed yield close to or above 1050 kg/ha and mean protein content of 27%, as well as good adaptability and stability in different environments, as it was simultaneously indicated through two methodologies. Therefore, these inbred lines may represent a potential elite germplasm in cowpea breeding programs and/or in the releasing of new cultivars adapted to the semi-arid region.

Keywords: Genotype-environment interaction; human nutrition; nutritional value; Semi-arid tropics; *Vigna unguiculata*.

Abbreviations: DSUG_Genotypes with determinate and semi upright growth; ISCG_Genotypes with indeterminate and semi-climbing growth; G×E_Genotype×environment interaction; WHO_World health organization.

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is a strategic pulse crop in semi-arid tropical regions, mainly in geographic sites where there is lack of alternative protein sources to local human populations, due to its natural ability to withstand water stress and to the protein content in its seeds (Vasconcelos et al., 2010). In addition, cowpea seeds are also a rich source of calories as well as of essential minerals and vitamins (Iqbal et al., 2006; Santos and Boiteux, 2015). The total seed protein content range in unimproved cowpea germplasm is of approximately 22 to 25% (Boukar et al., 2011). Breeding activities focused on incrementing total seed protein content allow developing cowpea cultivars that reach total seed protein content of up to 30% (Singh, 2007). More recently, transgressive segregating cowpea lines showing mean protein value of 34% were identified in progenies derived from a series of contrasting crosses (Santos et al., 2012). The total soybean seed protein value (which is the major agricultural dietary protein source) is of approximately 40% (Iqbal et al., 2006).

According to FAO (2014), it is estimated that approximately 805 million people were chronically undernourished between 2012 and 2014 and that they did not have access to the daily protein and carbohydrate intake recommended by the Health World Organization (WHO).

Supplementing the diet with plant proteins is an alternative to improve the human nutritional status, since the adequate provision level of animal origin proteins is either difficult to achieve or very expensive to afford in some vulnerable geographic regions (Iqbal et al., 2006). Many plant breeding programs have expanded their goals through the combination of yield increase and crop nutritional factor improvement in order to address the challenge of diminishing human malnutrition and the chronic diseases associated to it (Sands et al., 2009). According to Santos and Boiteux (2013), the development of cowpea cultivars to achieve higher seed protein and mineral contents, as well as superior agronomic performance, may represent an important contribution to poor human nutrition diminishing in semi-arid regions.

Overall, adaptability is the capacity a given genotype has to take advantage of a given set of environmental conditions, whereas stability is the ability it has to show highly predictable performance even under quite distinct environmental conditions (Cruz and Regazzi, 1994). The genotype × environment interaction (G×E) estimates are very important information pieces to breeding programs throughout the selection of high-yield and nutritionally improved cultivars. The significant G×E interaction may due to inconsistent genotype performance if such interaction is

assessed under different environmental conditions. It impairs the releasing of new stable cultivars. The sound statistical methodologies are the most recommended approaches to estimate $G \times E$ interaction and to identify cultivars that present higher adaptability and stability levels (Becker and Leon, 1988; Huehn, 1990a; 1990b; Cruz and Regazzi, 1994; Flores et al., 1998). It is worth analyzing $G \times E$ interactions whenever they are found, as well as using the results to assess the potential strong adaptability of the studied inbred lines and cultivars (Kang, 1998). A wide range of biometrical methods used to estimate the phenotypic stability and to analyze $G \times E$ interactions has been proposed (Becker and Leon, 1988; Flores et al., 1998). The yield stability and adaptability of cowpea have been investigated through linear regression analyses (Mano, 2009; Nunes, 2014), as it was described by Eberhart and Russel (1966), as well as through nonparametric analyses such as those described by Lin and Binns (1988). On the other hand, studies estimating the adaptability and stability parameters of cowpea protein content are not available in the literature. Balesevic-Tubic et al. (2011) reported greater protein content variation in soybeans than grain yield in a set of genotypes assessed in six different environments. Arslanoglu et al. (2011) also reported significant $G \times E$ interaction in the protein content of eight soybean cultivars, but they did not estimate the adaptability and stability parameters.

The selection of superior cultivars through the combination of high seed yield potential and protein content, and its strong adaptability and stability under different environmental conditions will have a huge positive impact on the cowpea production-market chains in the semi-arid tropics. Accordingly, the main aim of the present study is to estimate the adaptability and stability parameters of total seed yield and protein content in cowpea genotypes in order to release new cultivars through the combination of these value-added attributes.

Results

Environment assessments

Significant differences between mean square grain yield and protein content treatments were observed in all the experiments, except for the grain yield in Acauã (Piauí State), Juazeiro (Bahia State) and Petrolândia (Pernambuco State) in the DSGU trials (Table 1). The trial assessments under in-farm conditions in Acauã-PI, Dormentes-PE, Limoeiro-CE, and Petrolândia-PE did not interfere in the assessment, since the variation coefficient of these trials was lower than 40% (Table 1). These in-farm assays also made it possible analyzing the genotypes under contrasting crop management conditions. The highest yields were observed under the environmental conditions found in Limoeiro-CE (Table 1) and it indicated that some lines have the potential to achieve yield greater than 3,000 Kg/ha when more technological crop management systems are adopted. The range between the greatest and the lowest mean square errors in the ISCG and DSUG experiments was lower than seven, thus indicating error variance homogeneity and matching conditions to pool data from all the assays and to analyze them in a factorial experimental design (Cruz and Regazzi, 1994).

The mean yield in irrigated trials were 84% and 45% greater than that estimated in the ISCG and DSUG rain-fed trials, respectively (Table 1). The present results meet those from previous studies reported by Santos et al. (2008). The mean protein seed values were very similar either under irrigated or rain-fed conditions (Table 1).

Adaptability and phenotypic stability parameters of grain yield and protein content in cowpea genotypes

The 'BRS Acauã' cultivars, which belong to the ISCG subgroup, as well as the 'BRS Carijó' and 'BRS Tapaihum' cultivars, in the DSUG subgroup, showed the highest grain yield (Tables 2 and 3). These cultivars were previously assessed in the same set of locations, in the present study (except for Limoeiro), and exclusively selected through grain yield and earliness (Santos et al., 2008). The 'C3R' and 'C3B' lines in the ISCG experiment showed grain yield similar to that of the control 'BRS Acauã' cultivar, and good adaptability and stability parameters, according to the methodologies by Eberhart and Russell (1966) and Lin and Binns (1988) (Table 2). The 'C2O' line, which belongs to the DSUG subgroup, also showed grain yield close to that of the control 'BRS Carijó' and 'BRS Tapaihum' cultivars, as well as good adaptability and stability parameters, according to both analytical methodologies herein employed (Table 3).

The lowest protein content mean values (approximately 25%) were observed in Juazeiro-BA and Petrolândia-PE, and they suggested poor soil fertility and/or the absence of native *Rhizobium* strains. Such strains could increase nitrogen availability and, consequently, increase the overall seed protein content (Marinho et al., 2014). The inbred 'C2R', 'C3S', and 'C3F' (in the ISCG subgroup) and the 'C2M' and 'C2I' (in the DSUG subgroup) lines presented approximate mean protein value of 30% (Tables 2 and 3), which was greater than those of the control 'BRS Acauã', 'BRS Carijó', 'BRS Tapaihum', and 'Canapu' cultivars (a landrace widespread cultivation in the Brazilian semi-arid region). The inbred lines presenting the highest protein contents showed the lowest grain yields in the ISCG and DSUG experiments, as general rule (Tables 2 and 3). For example, the 'C2M' and 'C2I' lines showed the lowest yields. Such results suggest the prominent "phenotypic cost" of protein trait in overall cowpea seed production, thus indicating that the selection of cultivars through the combination of high yield and protein contents will not be a straightforward task. However, the breakage of the herein assessed association was observed in a subgroup of genotypes. The 'C2R', 'C3S', 'C3F' and 'C2I' lines showed good adaptability and stability parameters of protein content, according to the methodologies by Eberhart and Russell (1966) and Lin and Binns (1988).

Discussion

The recommendation for crop cultivars presenting good adaptability and stability is essential in countries comprising diverse agro-ecological regions, distinct crop management systems and well-defined climatic seasons. Accordingly, knowledge about the $G \times E$ interaction is necessary to estimate the different responses of cultivars/lines to a wide range of conditions (Becker and Leon, 1988; Simmonds, 1991; Cruz and Regazzi, 1994). Legume crops (such as cowpea) provide a daily protein intake alternative to many populations in semi-arid regions given the high cost and/or the low availability of animal protein (Iqbal et al., 2006). Cowpea seeds are featured by protein levels higher than those of other drought-adapted crops such as cassava, sweet potato, and sorghum, which are traditionally grown by subsistence farmers in semi-arid regions worldwide (Jager, 2013). Boukar et al. (2011) reported greater amino acid/protein content variability in cowpea, and Santos et al. (2012) indicated that, the seed protein content in cowpea could be improved through the standard breeding methods, which are largely

Table 1. Site of evaluation, crop management conditions, coefficient of variation (CV), grain yield/production mean (MP) (kg ha⁻¹), protein mean content (MPr) (%), error mean square (EMS) and treatment mean square (TMS) of seven trials involving 21 cowpea genotypes of indeterminate and semi-climbing growth (ISCG) habit at a plant population density of 100,000 plants/ha and 23 genotypes of determinate and semi upright growth (DSUG) habit at a plant population density of 200,000 plants/ha. These assays were carried out in seven environmental conditions across four States (Piauí-PI; Pernambuco-PE; Bahia-BA, and Ceará-CE) of the Brazilian semi-arid region.

Location	Crop management conditions/Year	CV (%)	Grain yield average (kg ha ⁻¹)			Protein content average (%)		
			MP	EMS	TMS	MPr	EMS	TMS
ISCG genotypes								
Acauã-PI	Rain-fed/2011	30	684	42345	87189**	26.8	0.87	8.6**
Petrolina-PE	Irrigated/2011	18	1322	57712	306194**	27.9	0.31	6.0**
Petrolina-PE	Rain-fed/2011	24	727	32140	90050**	27.6	1.04	9.6**
Dormentes-PE	Rain-fed/2011	36	689	60272	355248**	27.4	0.76	7.3**
Limoeiro-CE	Irrigated/2010	15	2192	114883	278797**	27.2	0.16	7.3**
Juazeiro-BA	Irrigated/2011	43	729	97575	202060*	24.9	0.86	8.6**
Petrolândia-PE	Irrigated/2011	32	897	81818	259048**	25.1	0.33	9.3**
Irrigated Mean	-	-	1285	-	-	26.3	-	-
Rain-fed Mean	-	-	700	-	-	27.3	-	-
DSUG genotypes								
Acauã-PI	Rain-fed/2011	26	1087	78306	86308 ^{NS}	26.8	1.37	8.0**
Petrolina-PE	Irrigated/2011	15	1697	62900	121491*	26.7	0.43	9.3**
Petrolina-PE	Rain-fed/2011	23	734	28273	52592*	27.4	0.6	7.7**
Dormentes-PE	Rain-fed/2011	31	887	75526	653413**	27.4	1.34	8.2**
Limoeiro-CE	Irrigated/2010	15	2057	102030	398371**	26.7	0.24	7.3**
Juazeiro-BA	Irrigated/2011	36	806	83516	116106 ^{NS}	24.5	1.24	5.9**
Petrolândia-PE	Irrigated/2011	38	692	71214	98114 ^{NS}	24.8	0.28	11.8**
Irrigated Mean	-	-	1313	-	-	25.7	-	-
Rain-fed Mean	-	-	903	-	-	27.2	-	-

** , * , ^{NS} Significant at 1%, 5% and non-significant by F test.

Table 2. Estimates of adaptability and phenotypic stability parameters for grain yield and protein content of 23 cowpea genotypes with indeterminate and semi-climbing growth habit employing the Eberhart and Russell (1966) and Lin and Binns (1988) methodologies. These assays were carried out in seven environmental conditions across four States (Piauí-PI; Pernambuco-PE; Bahia-BA, and Ceará-CE) of the Brazilian semi-arid region with a plant population density of 100,000 plants/ha.

Genotypes	Grain Yield						Protein content					
	Mean (kg/ha)	Eberhart and Russell		Lin and Binns			Mean (%)	Eberhart and Russell		Lin and Binns		
		β_1	σ_{di}^2	P_{i-gen}	P_{i-fav}	$P_{i-unfav}$		β_1	σ_{di}^2	P_{i-gen}	P_{i-fav}	$P_{i-unfav}$
01-C2R	886	1.3*	37767*	344349	341302	260596	30.1	1.2 ^{NS}	0.07 ^{NS}	0.02	.	0.07
02-C3S	904	1.0 ^{NS}	16770 ^{NS}	305069	256547	195908	29.0	0.9 ^{NS}	-0.06 ^{NS}	0.71	0.82	0.41
08-C3F	883	0.8 ^{NS}	1281 ^{NS}	315024	448315	345567	28.7	0.7*	0.59**	1.59	2.00	0.55
06-C6P	1013	0.9 ^{NS}	-2161 ^{NS}	224767	237240	190859	27.6	1.1 ^{NS}	0.27*	3.39	3.49	3.14
18-C1V	1019	1.2 ^{NS}	64770**	243650	363006	284308	27.6	1.0 ^{NS}	0.70**	3.89	3.83	4.04
04-C3Q	1021	1.2 ^{NS}	40905*	219184	115656	116089	27.3	1.2 ^{NS}	-0.00 ^{NS}	4.23	3.98	4.87
10-C2C	968	1.3*	29413 ^{NS}	287946	245638	194252	27.3	0.8 ^{NS}	0.02 ^{NS}	4.43	5.02	2.97
07-C1M	959	1.4**	58313**	301820	345600	268625	27.1	0.8 ^{NS}	0.06 ^{NS}	4.72	5.23	3.44
03-C3M	1150	0.9 ^{NS}	-10959 ^{NS}	152055	178216	145716	27.0	1.3*	0.91**	5.2	4.54	6.88
20-T16-2R	1171	1.0 ^{NS}	69547**	131728	96759	100742	27.0	0.6*	0.88**	5.44	6.32	3.24
09-C3L	950	1.0 ^{NS}	-22570 ^{NS}	249086	270084	199241	27.0	1.3*	0.77**	5.62	4.85	7.55
11-C1T	901	1.1 ^{NS}	50964*	331871	342600	261708	26.8	0.8 ^{NS}	0.44**	6.11	6.65	4.74
05-C3B	1214	0.9 ^{NS}	21638 ^{NS}	144688	201687	186056	26.7	0.9 ^{NS}	0.26*	6.22	6.69	5.0
13-C4G	1023	0.7*	27058 ^{NS}	251814	383246	304870	26.6	0.9 ^{NS}	0.35*	6.62	7.28	4.99
14-C6A	883	0.8 ^{NS}	30778*	310255	414330	327579	26.4	1.2 ^{NS}	-0.07 ^{NS}	6.97	6.54	8.05
15-C2T	934	0.9 ^{NS}	6603 ^{NS}	293622	333083	250318	26.3	1.2 ^{NS}	0.15 ^{NS}	7.73	6.92	9.78
22-BRSPujante	1050	0.8 ^{NS}	185586**	226464	315477	240687	26.1	0.1**	0.95**	9.80	11.86	4.63
19-C4I	1102	1.0 ^{NS}	-4448 ^{NS}	171894	136489	121888	26.0	1.4**	0.61**	10.57	9.38	13.57
16-C3P	1055	0.7*	27301 ^{NS}	239393	352244	277838	25.2	1.7**	0.13 ^{NS}	12.75	10.39	18.65
17-C6D	990	0.9 ^{NS}	43453*	256330	271362	219778	25.0	1.6**	0.94**	14.12	12.13	19.10
21-BRSAcauã	1341	1.0 ^{NS}	112508**	63731	60427	65052	24.6	1.0 ^{NS}	0.56**	15.79	16.08	15.07
23-Canapu	1154	0.5**	175495**	184265	393071	324478	24.3	0.3**	0.49**	18.05	20.28	12.47
12-C3R	1228	0.8 ^{NS}	-6056 ^{NS}	125105	147645	141590	24.1	0.6*	0.57**	18.43	20.56	13.11
Overall Mean			1034						26.7			
MS G*E			209439**						2.1**			

** , * , ^{NS} Significant at 1%, 5% and non-significant by 't' tests.

Table 3. Estimations of adaptability and phenotypic stability parameters for grain yield and protein content of 21 cowpea genotypes of determinate and semi upright growth using the Eberhart and Russell (1966) and Lin and Binns (1988) methodologies. These assays were carried out in seven environmental conditions across four States (Piauí-PI; Pernambuco-PE; Bahia-BA, and Ceará-CE) of the Brazilian semi-arid region with a plant population density of 200,000 plants/ha.

Genotypes	Grain yield						Protein content					
	Mean (kg/ha)	Eberhart and Russell		Lin and Binns			Mean (%)	Eberhart and Russell		Lin and Binns		
		β_1	σ_{di}^2	P_{i-gen}	P_{i-fav}	$P_{i-unfav}$		β_1	σ_{di}^2	P_{i-gen}	P_{i-fav}	$P_{i-unfav}$
17-C2M	893	0.9 ^{NS}	-35771 ^{NS}	479205	460190	486811	30.9	0.2**	-0.12 ^{NS}	0.06	0.08	.
04-C2I	953	0.9 ^{NS}	-53143 ^{NS}	404583	403348	405077	29.5	1.3 ^{NS}	0.23 ^{NS}	1.91	0.67	5.02
11-C2S	1196	0.9 ^{NS}	-45261 ^{NS}	276386	212120	302092	27.3	1.1 ^{NS}	0.02 ^{NS}	7.56	5.45	12.85
08-C1J	1121	1.0 ^{NS}	-38895 ^{NS}	249457	210761	264935	27.1	0.8 ^{NS}	-0.05 ^{NS}	8.50	7.63	10.67
02-C1R	1044	0.9 ^{NS}	-35616 ^{NS}	372225	284631	407263	26.9	1.1 ^{NS}	-0.21 ^{NS}	9.21	6.57	15.81
16-C1I	1078	0.8 ^{NS}	-26876 ^{NS}	353772	424630	325429	26.7	1.2 ^{NS}	-0.52 ^{NS}	10.28	7.42	17.43
06-C1S	1107	0.9 ^{NS}	-28073 ^{NS}	344578	283321	369081	26.5	0.9 ^{NS}	-0.43 ^{NS}	10.53	8.55	15.50
03-C3O	1203	0.4*	-5183 ^{NS}	235209	479624	137443	26.4	1.4 ^{NS}	0.05 ^{NS}	11.92	7.81	22.19
01-C1N	1171	1.2 ^{NS}	-55352 ^{NS}	269488	110202	333203	26.0	1.2 ^{NS}	-0.43 ^{NS}	13.44	10.20	21.54
09-C1F	1111	1.1 ^{NS}	-45872 ^{NS}	322260	169606	383321	26.0	1.1 ^{NS}	1.55**	14.31	11.72	20.78
12-C2B	1009	0.6 ^{NS}	71955 ^{NS}	496665	706628	412680	26.0	1.6*	0.56 ^{NS}	14.46	8.74	28.76
10-C2O	1243	1.1 ^{NS}	-46357 ^{NS}	232238	119297	277415	25.9	0.7 ^{NS}	-0.63 ^{NS}	13.71	12.54	16.62
07-C2J	1129	1.2 ^{NS}	43212 ^{NS}	202587	126085	233188	25.9	0.7 ^{NS}	-0.36 ^{NS}	13.88	12.30	17.85
14-C2Q	1032	1.0 ^{NS}	-46745 ^{NS}	364971	305326	388829	25.9	1.1 ^{NS}	-0.27 ^{NS}	14.08	11.35	20.91
13-C2A	1111	1.2 ^{NS}	-25378 ^{NS}	329596	102366	420488	25.9	1.2 ^{NS}	-0.17 ^{NS}	14.21	11.42	21.21
05-C1G	1129	1.0 ^{NS}	-42325 ^{NS}	304343	199124	346430	25.9	1.4 ^{NS}	-0.14 ^{NS}	14.69	10.95	24.04
15-C1O	1022	0.8 ^{NS}	-57381 ^{NS}	367170	429704	342156	25.8	0.6 ^{NS}	-0.18 ^{NS}	14.56	13.26	17.82
21-Canapu	1211	0.5*	493790 ^{NS}	228627	589931	84105	25.4	0.4 ^{NS}	-0.43 ^{NS}	16.17	15.59	17.65
19-BRS Carijo	1344	0.9 ^{NS}	-893 ^{NS}	135731	129146	138366	25.0	1.1 ^{NS}	0.37 ^{NS}	19.13	15.75	27.56
20-BRS Tapaihum	1323	1.4 ^{NS}	-15169 ^{NS}	123155	17749	165317	24.5	0.8 ^{NS}	-0.63 ^{NS}	21.77	19.44	27.59
18-18Marrom	1307	1.4 ^{NS}	6935 ^{NS}	185480	61935	234898	23.8	0.6 ^{NS}	0.03 ^{NS}	26.57	24.9	30.53
Overall Mean			1130						26.4			
MS G*E			200801**						2.01**			

**, *, ^{NS} Significant at 1%, 5% and non-significant by 't' tests.

used in self-pollinated crops, even under polygenic control. Cowpea cultivars with 30% protein content have been released in the African continent (Singh, 2007). More recently, Santos and Boiteux (2013) reported the development of lines presenting seed protein content of up to 34%, and it suggested the real opportunity to increase the protein content in this strategic plant species through traditional breeding.

Genotype × environment (G × E) interaction studies focused protein content are very scarce, even when it comes to important commodities such as soybean. So far, there are no such studies using cowpea. Therefore, our study is the first report in this crop species.

Multi-local trials play a major role in the identification of genotypes that combine high grain yield and protein content, and good agronomic performance in a wide range of contrasting environments, such as those under irrigated and rain-fed conditions. Many statistical approaches have been proposed in order to interpret significant G × E interactions and to identify genotypes presenting good adaptability and stability in different environments. The linear regression methodology proposed by Eberhart and Russell (1966) and the non-parametric one proposed by Lin and Binns (1988) have been largely applied to G × E studies. The low correlation levels have been observed between these methodologies (Silva and Duarte, 2006), and it suggests that one methodology cannot replace the other and that they should be applied together, as it was done by Pereira et al. (2009). The non-parametric methodology assumes some assumption violations, such as normality.

The greater protein contents reported in the current study are close to the value of cultivars released by IITA (Singh, 2007), in the African continent. The 'C2R', 'C3S', 'C3F', 'C2M' and 'C2S' lines showed protein content close to that of

previous assessments done with them; except for 'C2R', which showed mean protein content of 34.1% (Santos and Boiteux, 2013). The results reported in our study indicated that the selection and releasing of lines that combine high protein content and grain yield will not be a straightforward task, because the lines presenting the highest protein contents also showed the lowest yields in our assays. Santos and Boiteux (2013) reported non-significant phenotypic correlation between these two variables, and it did not meet the present findings. Mello Filho et al. (2004) reported similar negative correlation between grain yield and protein content in eight soybean populations. Wilson (2004) suggested the independent manipulation of these two variables, and it could be achieved through the selection of individual lines that present either intermediate grain yield and protein content values or mean values above that of the experimental means. The 'C3Q', 'C3M', 'C2S', and 'CIJ' lines showed yield equal to or above the experimental means, as well as protein content above that of the experimental means and good adaptability and stability through the methodologies by Eberhart and Russell (1966) and by Lin and Binns (1988) (Tables 2 and 3). These lines could be strong candidates to be released as new cultivars in the semi-arid regions. High protein content lines such as 'C2R', 'C3S', 'C2M' and 'C2I' could be employed in backcross breeding programs; 'C3Q', 'C3M', 'C2S' and 'CIJ' can be used as recurrent parents in order to simultaneously increase yield and protein content, as it was suggested by Wilcox and Cavins (1995) in their study on soybeans.

Cowpea is broadly grown in semi-arid regions due to its tolerance to water stress and substantial grain yield in comparison to other legumes such as common beans, lentils, and chickpeas. Accordingly, the selection of superior cultivars through the combination of high yield and seed

protein content, and good adaptability and stability under different environmental conditions will have a huge positive impact on cowpea production-market chains, mainly in semi-arid tropical regions where there is lack of protein sources available for human consumption. The 'C3Q', 'C3M', 'C2S' and 'CIJ' lines identified in the present study, after their assessment under irrigated and rain-fed conditions, showed mean protein value of 27%, which is higher than that observed in most of the current commercial cowpea cultivars (approximately 24%). These inbred lines may represent a potential elite germplasm in breeding programs focused on improving the protein levels to 30% or more, depending on the line and/or on the direct release of new nutritionally improved cultivars adapted to the Brazilian semi-arid region.

Materials and Methods

Plant materials

The cowpea lines assessed in the present study were developed and selected through high protein content and grain yield of crosses involving inbred lines introduced by the International Institute for Tropical Agriculture (IITA) to Brazilian-adapted cultivars, as it was previously described (Santos and Boiteux, 2013). The cowpea genotypes were subdivided in two different agronomic groups. Experiment #1 was composed of a set of genotypes presenting indeterminate and semi-climbing growth (ISCG) habits, with 23 treatments (20 inbred lines) and three commercial cultivars were used as control. Experiment #2 was composed of a set of genotypes presenting determinate and semi upright growth (DSUG) habits, with 21 treatments (17 inbred lines), and four cultivars were used as control. The experiments were carried out with no nitrogen fertilization and *Rhizobium* inoculation. It was intentionally done, since the *Rhizobium* inoculation may increase protein content due to its effects on nitrogen availability (Marinho et al., 2014). The experiments were carried out in different locations in the States of Bahia, Ceará, Pernambuco, and Piauí, which are located in the Northeastern region of Brazil (Table 1). The experimental design was a completely randomized block with three repetitions. Three field assays were carried out under irrigated management conditions (in the second semester of the year) and four assays were conducted under rain-fed conditions (in the first semester of the year). Each experimental net unit measured 3 m², and the plots measured 3.0 × 2.0 m. The plant spacing was 1.0 m × 0.1 m and 0.5 m × 0.1 m; plant density per hectare was 100,000 and 200,000 in the assays involving the ISCG and DSUG genotypes, respectively.

Protein quantification

The flour samples were obtained from the mature seeds (10 g sample) of each plant. They were ground in a MA 630/1 mill (Marconi, Brazil) for 1 min, using a 35-mesh screen. The flour samples were analyzed (in duplicate) according to the method No. 920.87 of the Association of Official Analytical Chemists (AOAC, 1995). The finely ground and dried sample (0.2 g) was weighed in a micro-Kjeldahl flask containing the catalyst solution plus 5 mL H₂SO₄ (concentrated). The flasks were placed in a bank of heaters to be digested (temperature equal to 350°C) in fume hood for four hours until a clear solution was observed in the tube. The digested solution was placed in the Kjeldahl apparatus and 15 mL of 50% (w/v) NaOH was added to each tube. The mixture was then steam distilled and the ammonia released from the process was collected in a 100 mL conical flask containing 10 mL 2%

boric acid plus a mixed indicator solution. The green reaction solution was titrated in 0.01 M HCl solution. At the end point, the green color turned pink, and it indicated that all the nitrogen trapped as ammonium borate was removed as ammonium chloride. The nitrogen percentage was calculated through the formula: % N = [0.14 * correction factor for HCl solution * total volume of titrated HCl]. The crude protein amount was determined through the multiplication of the nitrogen percentage using the constant factor 6.25. The total protein was corrected to dry matter, which was obtained after all the samples were dried at 106°C for 12 h. All the analyses were performed at the Animal Nutrition Laboratory in Embrapa Tropical Semi-arid.

Statistical analyses

Experimental data about the location and those pooled in the seven environments were analyzed through the GLM procedure and through the Ls mean options of the SAS (SAS, 1989). The grain yield by location was adjusted to the mean number of plants in the experimental units by the covariance method using a SAS code, as it was described by Vencovsky and BARRIGA (1992). The adaptability and stability parameters were estimated according to the methodologies by Eberhart and Russell (1966) and Lin and Binns (1988), which are available in the Genes software (Cruz, 2006). According to the methodology described by Eberhart and Russell (1966), the regression coefficient is associated with a linear component, thus suggesting genotype adaptability: $\beta_i=1$ indicates broad adaptability, whereas the regression deviation equal to zero ($\sigma^2_{di}=0$) indicates good stability. The P_i gen parameter describes the genotype stability, which was defined as the mean square of the distance between genotype i and the genotype presenting the strongest response in the methodology by Lin and Binns (1988). The best genotypes are those showing the lowest P_i gen parameter values. In addition, the P_i .fav and P_i .unfav stability parameters were estimated according to Carneiro (1998). These parameters provide information about favorable and unfavorable environments. Genotypes presenting low values are those with the best performance in such contrasting environments.

Conclusion

The inbred lines showing the highest protein contents presented the lowest grain yields, thus indicating the prominent "phenotypic cost" of protein trait in overall cowpea seed production. However, the breakage of the herein studied association was observed in a subgroup of inbred lines such as 'C3Q', 'C3M', 'C2S', and 'CIJ'. These lines showed yield approximate to or above 1050 kg/ha, mean protein content of 27%, good adaptability and stability in different environments, as it was simultaneously indicated by the two methodologies. Therefore, these inbred lines may represent a potential elite germplasm in cowpea breeding programs and/or in the releasing of new cultivars adapted to the semi-arid region.

Aknowlegments

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