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Chapter 24

Cowpea Breeding for Drought Tolerance — From Brazil to World

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/62000

Abstract

The aims of this chapter are to (i) present the importance of the cowpea crop, (ii) demonstrate problems related to drought, (iii) describe aspects related to flower structure and hybridization, and (iv) reveal how the genotype selection with tolerance to water deficit will promote increase of the yield in this culture. This chapter describes that Nigeria, Niger, and Brazil are the leading producers of cowpea crop worldwide, and this crop has a large influence on the economy of these countries. However, the drought problem can be frequently observed in areas with agricultural potential, with negative effects on production components and a consequent decrease of the yield in this culture. Breeding programs aimed at drought tolerance using selection strategies linked to genetic, biochemical, physiological, nutritional, and agronomic characteristics can help increase the yield and reduce losses promoted by the drought. In addition, flower structure and hybridization technique used in Núcleo de Pesquisa Vegetal Básica e Aplicada (NPVBA/UFRA) are presented, as well as populations are evaluated and plant management are explained in detail. This chapter describes the results obtained in other breeding programs aimed at drought tolerance and also explains the potential uses to increase the crop yield.

Keywords: Vigna unguiculata, production, drought, breeding

1. Introduction

The cowpea [*Vigna unguiculata* (L.) Walp.] is cultivated widely in several countries due to its social, economic, and nutritional importance [1]. The aim of growing cowpea crop in such a huge quantity is to market its grains. These species are abundant in the regions of Latin

America, Africa, and Asia, while Nigeria, Niger, and Brazil are emerging as the leading producers of cowpea [2].

In Brazil, the production of cowpea is concentrated in the North and Northeast regions, because it presents interesting metabolic characteristics, such as low nutrient requirements, less water consumption, and well adapted to tropical environments, compared to other legumes [3-4]. Until the 1980s, the cowpea was almost exclusively grown by small and medium farmers. Currently, this culture seems to occupy other agricultural scenarios in areas with high irrigation potential, or/and also during the off-season; next only to the soybean crop, cowpea crops are being exploited by large producers by adopting new technologies [5-6].

The plants of various species under field conditions are constantly exposed to abiotic or biotic stresses, and suffer interactions that can significantly affect their performance [7], including the culture of cowpea [8]. Lawlor [9] points out that the main limiting abiotic factors of productivity in various plant species are drought, flooding, low and high temperatures, salinity, excess radiation, toxic heavy metals, and excessive macro- or micronutrients [10]. Drought is considered a major cause of the reduction of global agricultural production [11-12].

Drought is a frequently observed event and recurrent in areas with agricultural potential, mainly in tropical regions, causing an increase in temperature and decrease in the relative humidity [13]. This stress can be caused by irregularities in the distribution of rainfall [14-15] and/or inappropriate supplement irrigation for crops [16-17].

Thus, the water deficit acts as a limiting factor for both quality and production in several species with agronomic potential [18-20], including the cowpea [21]. The occurrence of drought during the growing and development and reproductive and maturity stages usually results in lower growth rates and development [22], flower abortion [23], and reduced grain production (GP) [24], thus complicating the reproduction process in the cowpea crop.

The decrease in the growth and development in cowpea plants observed during vegetative stages is related to negative interference periods of drought, which is caused by the lower weight of the aerial part of the plant [25], reduction in the expansion rate of leaf area [26], and severe reductions in gas exchange [27].

Drought induces molecular, physiological, biochemical, and morphological changes in cowpea plants [28-31], which are considered as adaptation strategies and survival mechanisms of the species [32]. Another problem normally connected to drought is the rise in temperature in the plant tissues due to reduced transpiration rate and consequent loss of effectiveness of thermoregulatory mechanism [33, 34]. This is due to water stress, as water is an essential element for the growth and development of all species of plants and plays an important role in photosynthesis, transport of organic solutes, and temperature control [35].

The increase in the rate of growth of the root system is a possibility to overcome the water stress in plants [36]; for, under such conditions, the stimulation of root growth in depth and the wetter areas of the soil profile [18] adaptations can check and control drought [26]. The root system of common bean often lies in surface when water availability is adequate [37]. Guimarães et al. [38] observed that the genotypes more tolerant to water stress had more

http://dx.doi.org/10.5772/62000

developed root systems compared with the susceptible genotypes. Additionally, Peña-Valdivia et al. [39], investigating common bean plants, describe negative changes related to the anatomy of the root, such as reductions in the xylem vessel and cross-sectional area of the root, in domesticated plants, while wild plants remained unchanged in structures and showed greater tolerance to drought.

The osmotic adjustment is another mechanism that can directly contribute to water retention in the plant tissue [40], for increasing the tolerance to dehydration [34]. This adjustment is accomplished by substances called osmoregulators such as carbohydrates and amino acids [41-42]. The accumulation of metabolites of plants provides a decrease in osmotic potential (ψ_s) via a net increase in intracellular solute [43], which induces higher water retention in the tissue [44], and thus can control the plant cell elongation and expansion in growth regions [45].

The type of substances used in osmotic adjustment depends on the species and tissue [46]. However, the degree of adjustment depends on the speed at which the water deficit is applied, and osmotic adjustment is directly proportional to the intensity of water deficit [47]. The proline and glycine betaine are usually amino acids that act as osmotic adjusters in cowpea plants exposed to water deficit [48-49]. In water stress conditions, an increase in the synthesis of abscisic acid (ABA) in the roots is also observed, which is then transported to the shoot via the xylem [50].

In cowpea plants, overexpression of genes *CPRD 8*, *CPRD12*, *CPRD14*, *CPRD22*, and *CPRD46* conferring tolerance to water stress [28], as well as *Vuc*APX genes, *Vup*APX, and *Vut*APX-connected *Vus*APX production of antioxidant enzymes [51], are reported, besides the high-level expression of *PvP5CS* gene associated with the production of proline, an amino acid that holds the osmotic adjusting function across species during drought.

The responses of cultivars and lines in cowpea are different in relation to water stress during the cycle [52]. In addition, there are factors such as the intensity and duration of drought that can intensify such stress [53-55].

The culture of cowpea requires basic and applied research related to the impact caused by water deficit, due to frequent and severe loss of production related to abiotic stress [56-58] and the high economic and nutritional importance to the producers and grain-consuming countries, such as Brazil [59-60].

The selection of genotypes of tolerance to drought has been carried out in several breeding programs [61-62]. However, the large number of genes involved in tolerance to drought [63], combined with the influence of the environment, hinders the selection of plants in segregating generations [53,57] and affects the evaluation lines/cultivars by virtue of the interaction, provided genotype environment often is significant [64].

The crosses between contrasting parenting have been widely used and allow to investigate the genetic control of quantitative traits such as drought tolerance [15,54]. Therefore, the characteristics described by Bastos et al. [8] are present in cultivars of cowpea, BRS Paraguaçu (drought tolerance) and Tracuateua-192 (sensitivity to drought), and are suitable for the purpose of this research and justify the hybridization between these cultivars, which are contrasting in relation to tolerance to drought.

Adequate selection methods used in breeding programs of a species can reduce time and investment and maintenance of evaluating plants that could be previously discarded [65]. Thus, selection strategies linked to genetic, biochemical, physiological, nutritional, and agronomic characteristics can help due to the high detection efficiency and low cost [66]. In addition, the knowledge of the genetic control related to these characteristics will establish breeding strategies and selection of agronomically superior plants, with genes of tolerance to drought.

2. Objectives

The aims of this chapter are to (i) present the importance of the cowpea crop, (ii) demonstrate problems related to drought, (iii) describe aspects related to flower structure and hybridization, and (iv) reveal how the genotype selection with tolerance to water deficit will promote increase of the yield in this culture.

3. Flower structure in cowpea

In relation to flowers, the cowpea bean flower is hermaphrodite, deciduous, cyclic, dichlamydeous, and heterochlamydeous with zygomorphic symmetry (Figure 1). The five sepals and petals present are in free condition, denominated polysepalous and polypetalous, respectively [67].

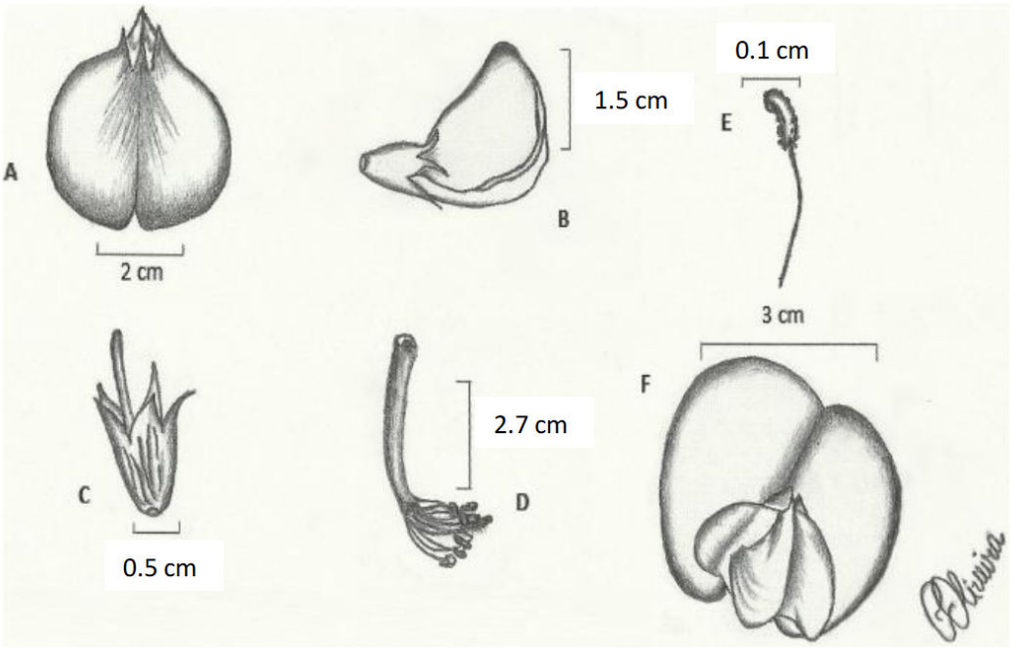

Figure 1. *V. unguiculata* floral pieces. (A) standard; (B) post-anthesis flower structure; (C) sepals; (D) stamen and pistil; (E) longer stamen; (F) keel formation [67].

Their corolla is papilionaceous; that is, it has an upper petal called standard, two side petals called wings and two lower, inner petals jointly called keel (Figure 1). One of the stamens is longer than the other nine (heterostemonous). Stamens are free, being diadelphous, with simple ramification; the anthers are free and basifixed, enclosed in relation to the corolla, longitudinally dehiscent and introrsed [67]. They are multicarpellary, syncarpous, with insertion in the terminal style. The hilum, chalaza, and micropyle are in the same straight line; that is, their ovule is classified as orthotropous [67].

4. Aspects linked to artificial hybridization

The results described by Ribeiro et al. on aspects of the floral biology of *Vigna unguiculata* reveals that anthesis occurred between 05:00 and 05:30 h [67]. In pre-anthesis, at 4:00 h, the greenish flowers remained. In the stigma receptivity test, peroxidase action was observed at all times. As noted by Rocha et al., the floral opening of cowpea bean flowers begins around 05:30 h and continues until 09:30 h, when the stigma is still receptive (Figure 2) [68].

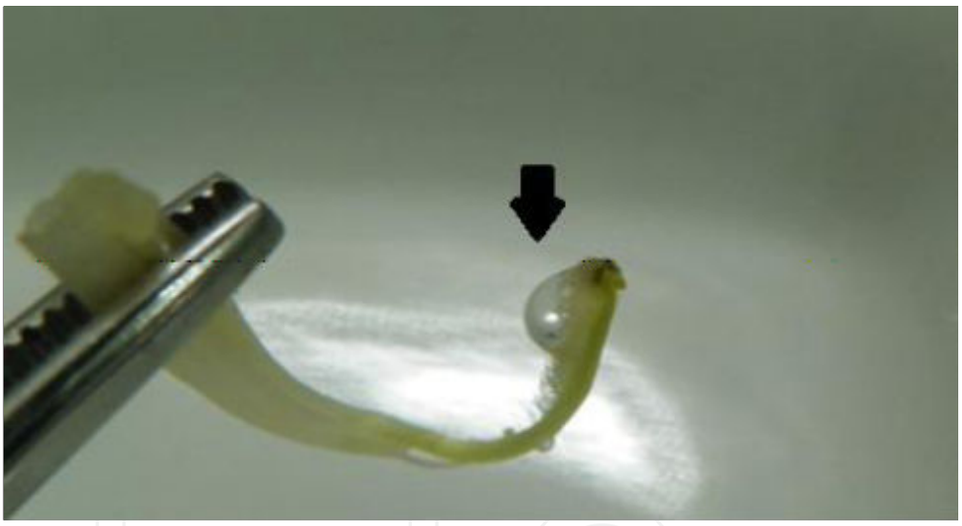

Figure 2. Peroxidase activity on *V. unguiculata* stigma, demonstrating receptiveness [67].

5. Adaptations to hybridization in Brazilian conditions

The method of artificial hybridization described by Rachie et al. [69], as well as Zary and Miller Jr [70], was adjusted to conditions of infrastructure from Núcleo de Pesquisa Vegetal Básica e Aplicada (NPVBA) of the Universidade Federal Rural da Amazônia (UFRA) and climatic conditions of Northern Brazil (Figure 3), which used flower pollen collected in the morning (between 06:00 and 08:00 h) and stored in the refrigerator until use.

In the evening, the flower buds were emasculated and pollinated (16:30 and 17:30 h) (Figure 3). This method provided a higher percentage of successful pollination. This result is in

agreement with the Zary and Miller Jr [70] method, described by Teófilo et al. [71], who reported that the success of this method could be because the surface of the emasculated flowers are more receptive to pollen grains in the late afternoon, since, in this period, the temperature and moisture conditions are more appropriate.

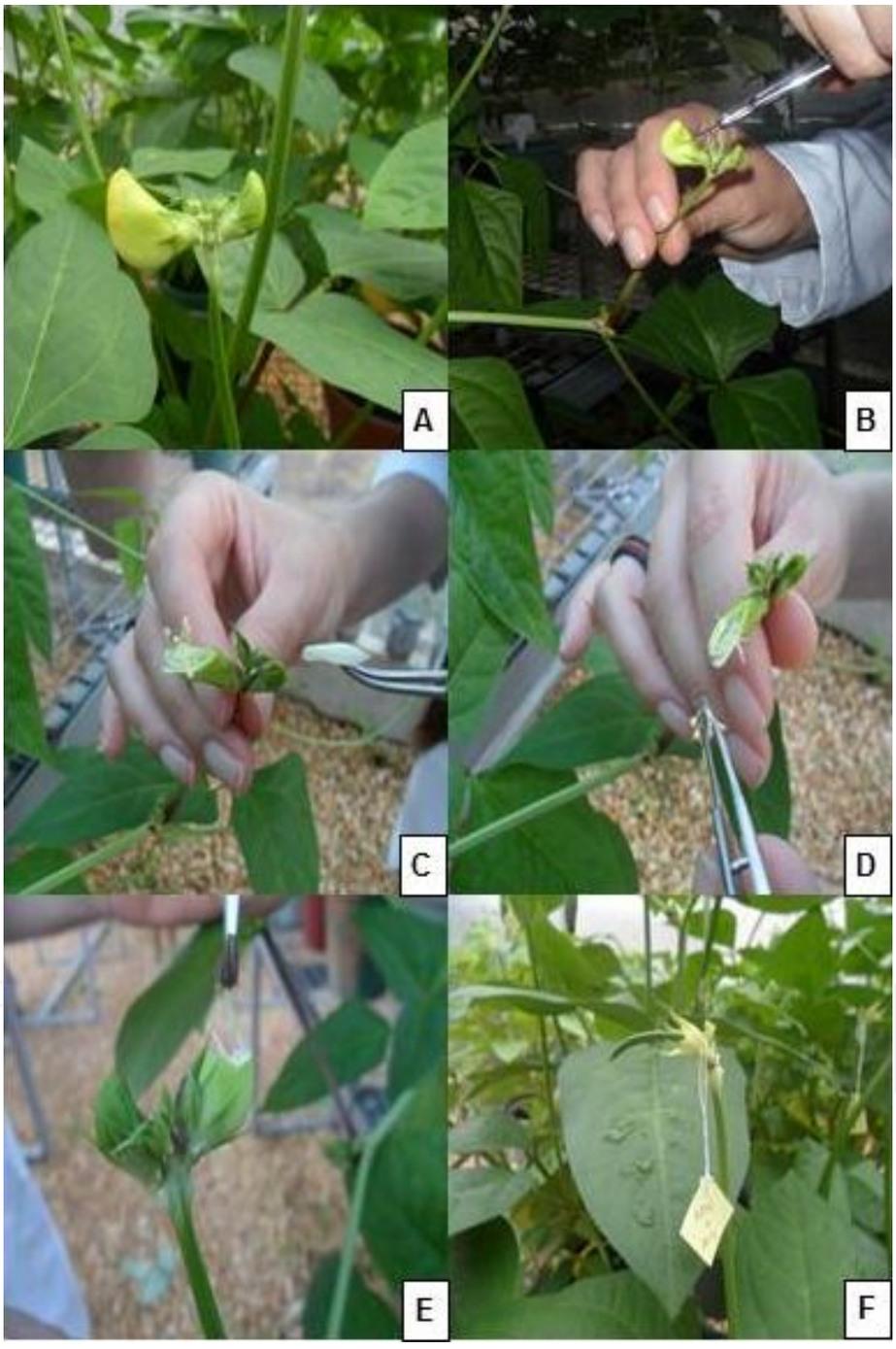

Figure 3. Greenish flowers with flower from left in pre-anthesis stage (A); cut of standard in medium region (B); removal of the standard (C); removal of the anthers (D); application of pollen viable (E); successful pollination and pod formation (F).

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6. Populations evaluated and plant management

After hybridizations (Figure 4), the hybrid seeds were multiplied to obtain the F_2, BC_1, and BC_2 generations. In each pot, only one seed was placed. In this study, containers with capacity of 3 L containing holes in bottom were used, aiming to drain the excess water. The substrate was composed of a mixture of Plantmax® and sand in 3:1 ratio (v/v). Fertilization was performed according to exigencies of cowpea crop and previous substrate analysis, with the fertilization applied at regular intervals of 15 days until the 45th day after implementation experiment.

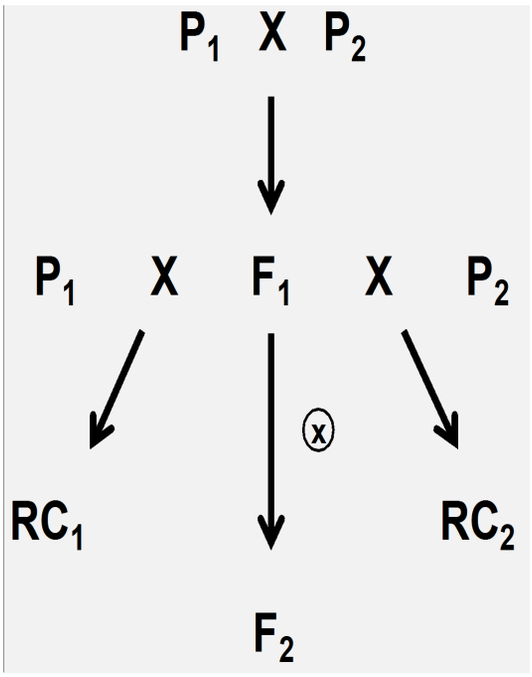

Figure 4. Scheme to obtain six populations (P_1, P_2, F_1, F_2, BC_1, and BC_2) from crosses between LP 97-28 (low tolerance to drought) × IPR-Uirapuru (high tolerance to drought).

7. Irrigation and water-deficit application

All plants were irrigated every day for 15 minutes at 10:00 and 13:00 h. The irrigation within the greenhouse was performed by a microsprinkler system, with a flow rate of 10 L h^{-1}. The three cycles of moderate stress were induced by irrigation suspension for four days at 25, 35, and 45 days after seedling emergence, whose periods coincided with the phenological stages V_3, R_6, and R_8, respectively. In the cowpea crop, these periods described are related to vegetative, flowering, and pod filling stages, respectively (Figure 5).

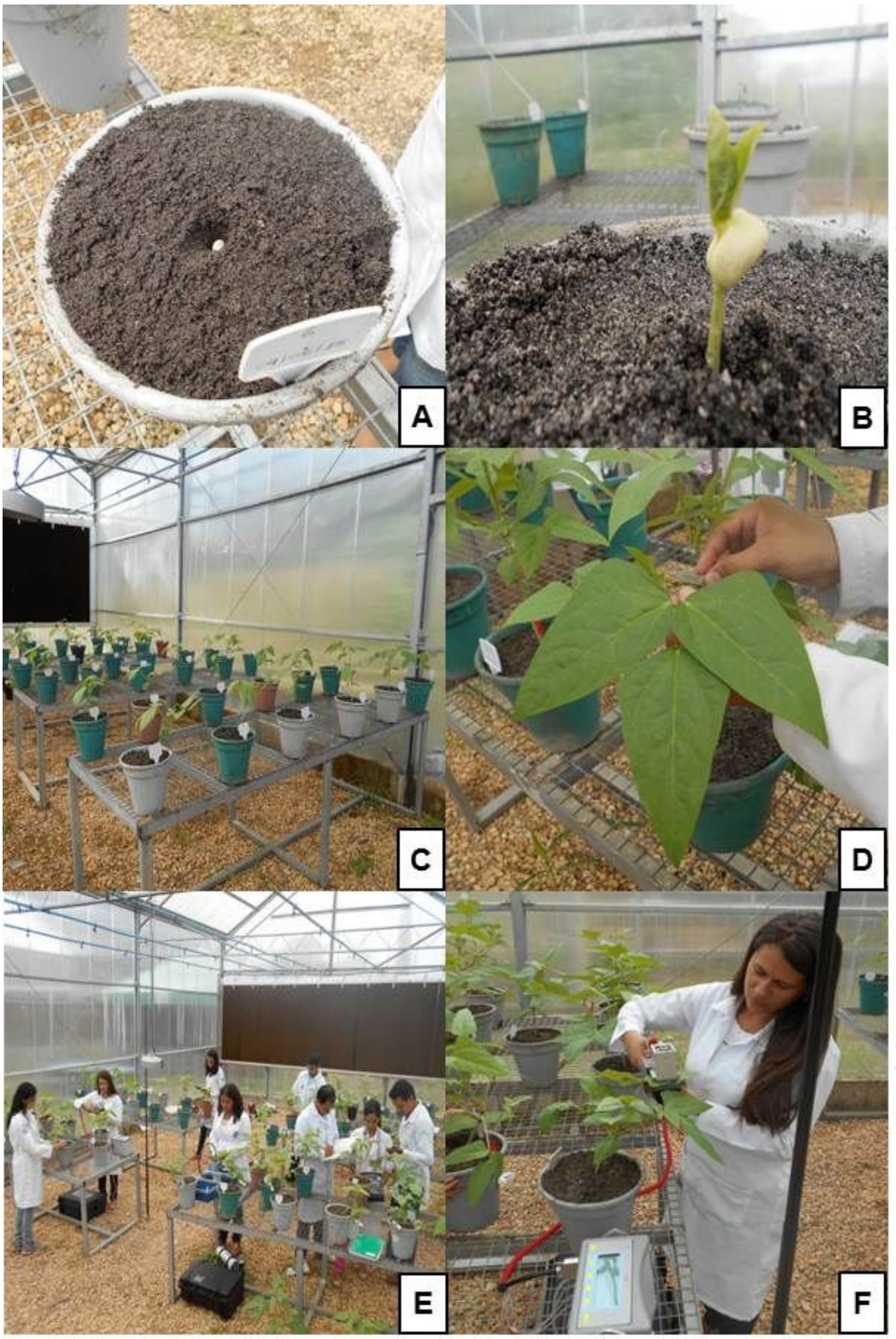

Figure 5. Seed placed into substrate (A); *V. unguiculata* seedling (B); plants with 14 days (C); trifoliate leaf (D); data obtained during experiment (E); infra-red gas analyzer (F).

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8. Results obtained in breeding programs aimed at drought tolerance

The study was conducted by Lobato et al. [72] with six populations, with two parents P_1 and P_2, and F_1, F_2, BC_1, and BC_2 generations, derived from the cross between LP 97-28 (low tolerance to drought) × IPR-Uirapuru (high tolerance to drought). Regarding production components, the grain production (GP) results reveal that the evaluated plants had values between 0.01 and 9.78 g. The low and high means for all populations were 2.30 and 6.86 g, respectively (Table 1). The F_2 generation showed the best performance. The low and high values for variance were obtained in the parental P_1 (1.51) and F_2 (8.88) generations, respectively. In relation to the average weight of 100 seeds (W100s), the values ranged between 12.56 and 29.64 g. In addition, the low and high means were 18.49 and 21.83 g, with the greatest means observed in the F_1 and P_2 populations (Table 1). The greatest variance of 11.12 was obtained in the F_2 generation. For the number of pods per plant (NPP), the plants studied had values between 1 and 19, and the lowest and highest means were 2.70 and 7.02, respectively (Table 1). The best result was found in the F_2 generation, while the lowest value was observed in the BC_1 generation. The highest variance of 8.08 was observed in the F_2 generation (Table 1). For the number of seeds per pod (NSP), the plants collected in this study had values between 1.0 and 6.8. The low and high means were 4.17 and 4.64 in populations P_1 and BC_2, respectively (Table 1). Additionally, the best result was found in generation BC_2. The low and high variances were 0.38 and 1.54 (Table 1) and were obtained in the P_2 and F_2 generations, respectively [72].

The estimated means of the segregating generations and the existence of high genetic variation (σ^2) coupled with additivity indicated the presence of transgressive individuals. These findings enabled the selection of promising genotypes for drought tolerances higher than those of IPR-Uirapuru and LP 97-28, the parent lines in this study. In terms of the estimated variances in the study populations, the best performances were observed for the segregating generations (F_2, BC_1, and BC_2), which demonstrated higher values for all traits compared to the parent (P_1 and P_2) and F_1 generations (Table 1). These results can be attributed to the large segregation of genes and, consequently, the higher amplitude in the distribution of the drought stress tolerance values, indicating genetic variation for the evaluated traits [73]. Similar results to those found in this study in terms of the GP of the F_2 generation were reported by Szilagyi [74] for experiments with the common bean grown under adequate conditions (irrigation) and drought stress. This author studied production components in six populations derived from crosses between F332 and Ardeleana.

Higher variances for GP, W100s, NPP, and NSP were observed in the F_2 generation, revealing greater plant heterogeneity and suggesting great variability within this population. Genetic variability is extensively explored in breeding programs; it serves as the basis for selection and provides opportunities to establish a desired characteristic [75]. Smaller variances were obtained in the parents, confirming homozygosis in these populations due to the line and cultivar.

Regarding genetic control, the values of the phenotypic (σ_p^2), environmental (σ_e^2), genotypic (σ_g^2), additive (σ_a^2), and dominance variances (σ_d^2) for grain production were 8.88, 2.97, 5.91, 5.75, and 0.16, respectively (Table 2). The genotypic variance corresponded to 66.6% of the phenotypic variance (total), and the additive variance accounted for 97.3% of the genetic variance. In W100s, the phenotypic, environmental, genotypic, additive, and dominance variances were 11.12, 2.55, 8.57, 7.66, and 0.91, respectively (Table 2). For this characteristic, the genotypic variance accounted for 77.1% of the total variance, while the additive variance corresponded to 89.4% of the existing genetic variance. In relation to NPP, the phenotypic, environmental, genotypic, additive, and dominance variances were 8.08, 2.33, 5.75, 4.73, and 1.02, respectively (Table 2). The phenotypic variance accounted for 71.2% of the genetic variance. Additionally, the additive variance corresponded to 82.3% of the genotypic variance. For the NSP, the phenotypic, environmental, genotypic, additive, and dominance variances were 1.54, 0.56, 0.98, 0.76, and 0.22, respectively (Table 2). The genotypic variance represented 63.7% of the phenotypic variance in this characteristic. The additive and dominance variances contributed to 77.6 and 22.4% of the genotypic variance, respectively [72].

The results indicate high contributions of additive variances in relation to genotypic variance and intense additive allelic interactions on all the evaluated traits. The existence of high additive variance suggests the identification of superior genotypes [76]. Typically, breeding methods that take advantage of high additive variance to obtain genetic gains are more important for the improvement of autogamous species, such as *Phaseolus vulgaris* [73].

According to this research, the use of additive variance is recommended as an indicator when studying GP, W100s, NPP, and NSP in the cross (LP 97-28 × IPR-Uirapuru), because it accounts for a significant portion of genotypic variance.

The estimates of broad-sense heritability (H^2 %) ranged between 63.6 and 77.0% (Table 2), and the high and low values were found in the W100s and NSP characteristics, respectively. The estimates of narrow-sense heritability (h^2 %) oscillated between 49.2 and 68.9% (Table 2), and the high and low values also corresponded to the W100s and NSP characteristics.

The average degree of dominance (add) values were 0.22, 0.48, 0.65, and 0.76 for the GP, W100s, NPP, and NSP characteristics, respectively (Table 2). The minimum number of genes (mng) that controlled the GP, W100s, NPP, and NSP characteristics were 4.7, 4.4, 8.6, and 5.5, respectively (Table 2).

The results related to broad- and narrow-sense heritabilities described in this study are high because studies involving populations are normally conducted under field conditions and high levels of environmental interference reduce genetic variances and produce lower heritabilities. Higher heritability coefficients may be caused by greater additive genetic variance, lower environmental variance, or minor interactions between genotype and environment [77]. Additionally, similar results for broad- and narrow-sense heritabilities indicate that the dominance effect is null. However, if the broad-sense heritability is higher than the narrow-sense heritability, the dominance effect is present [78].

LP 97-28×IPR-Uirapuru		GP(g)		W100s(g)		NPP		NSP	
	n	m	σ^2	m	σ^2	m	σ^2	m	σ^2
LP 97-28 (P_1)	16	4.60	1.51	18.49	1.19	5.69	1.29	4.17	0.68
IPR-Uirapuru (P_2)	18	4.48	1.89	21.83	1.37	4.39	2.13	4.60	0.38
F_1	7	4.19	4.25	21.83	3.18	4.43	2.95	4.29	0.59
F_2	166	6.86	8.88	21.29	11.12	7.02	8.08	4.16	1.54
BC_1	10	2.30	4.92	19.29	6.44	2.70	5.34	4.54	1.43
BC_2	39	3.31	7.07	20.52	8.13	3.44	6.09	4.64	0.88

Table 1. Number of evaluated plants (*n*), means (*m*), and variances (σ^2) from grain production (GP), average weight of 100 seeds (W100s), number of pod per plant (NPP), and number of seeds per plant (NSP) obtained in six populations (P_1, P_2, F_1, F_2, BC_1, and BC_2), derived from cross between LP 97-28 × IPR-Uirapuru, Maringá-PR, Brazil, 2011 [72].

LP 97-28×IPR-Uirapuru	GP	W100s	NPP	NSP
Phenotypic variance (σ_p^2)	8.88	11.12	8.08	1.54
Enviromental variance (σ_p^2)	2.97	2.55	2.33	0.56
Genotypic variance (σ_p^2)	5.91	8.57	5.75	0.98
Additive variance (σ_p^2)	5.75	7.66	4.73	0.76
Dominance variance (σ_p^2)	0.16	0.91	1.02	0.22
Broad sense heritability (H^2%)	66.4	77.0	71.1	63.6
Narrow sense heritability (h^2%)	64.7	68.9	58.5	49.2
Average degree of dominance (*add*)	0.22	0.48	0.65	0.76
Minimum number of genes (*mng*)	4.7	4.4	8.6	5.5

Table 2. Estimates of phenotypic variance (σ_p^2), environmental variance (σ_e^2), genotypic variance (σ_g^2), additive variance (σ_a^2), dominance variance (σ_d^2), broad-sense heritability (H^2 %), narrow-sense heritability (h^2 %), average degree of dominance (add), and minimum number of genes (mng) related to grain production (GP), average weight of 100 seeds (W100s), number of pod per plant (NPP), and number of seeds per plant (NSP) obtained in six populations (P_1, P_2, F_1, F_2, BC_1, and BC_2), derived from cross between LP 97-28 × IPR-Uirapuru, Maringá-PR, Brazil, 2011 [72].In differential of selection (DS), gain by selection (GS) and predicted genetic gain, the characteristics of grain production, average weight of 100 seeds, number of pods per plant, and number of seeds per pod had differential of selection (DS) values ranging from 1.49 to 4.85 (Table 3).

Characteristic	Genetic component					
	Mi	Ms	Ds	GS	GS(%)	PGG
GP	6.86	11.45	4.59	2.93	42.7	9.79
W100s	21.29	26.14	4.85	3.29	15.5	24.58
NPP	7.02	11.12	4.10	2.37	33.8	9.39
NSP	4.61	6.10	1.49	0.73	15.9	5.34

Table 3. Mean initial in F_2 generation (Mi), mean of selected plants in F_2 generation (Ms), differential of selection (DS), gain by selection (GS), gain by selection expressed in percentage [(GS (%)], and predicted gain genetic (PGG) related to grain production (GP), average weight of 100 seeds (W100s), number of pod per plant (NPP), and number of seeds per plant (NSP) obtained in six populations (P_1, P_2, F_1, F_2, BC_1, and BC_2), derived from cross between LP 97-28 × IPR-Uirapuru, Maringá-PR, Brazil, 2011 [72].

The high and low values were obtained for the W100s and the NSP, respectively. In relation to gain by selection (GS), the GP, W100s, NPP, and NSP characteristics had values of 2.93, 3.29, 2.37, and 0.73, respectively (Table 3). When expressed as a percentage (% GS), the grain production trait had the highest value for gain by selection at 42.7%. The lowest value was found for the average weight of 100 seeds. The predicted genetic gain (PGG) values were 9.79, 24.58, 9.39, and 5.34 for the GP, W100s, NPP, and NSP characteristics, respectively (Table 3) [72].

Characteristic	GP	W100s	NPP	NSP
GP	-	0.36	0.96**	0.01
W100s		-	0.13	0.14
NPP			-	-0.20
NSP				-

Asterisks (**) indicate significance to 0.01 of probability by the *t*-test.

Table 4. Coefficients of phenotypic correlation between grain production (GP), average weight of 100 seeds (W100s), number of pod per plant (NPP), and number of seeds per plant (NSP) obtained in six populations (P_1, P_2, F_1, F_2, BC_1, and BC_2), derived from cross between LP 97-28 × IPR-Uirapuru, Maringá-PR, Brazil, 2011 [72].

Regarding correlations between characteristics, results indicated that all characteristics were directly proportional (Table 4), except between the NSP and NPP, which were inversely proportional. Additionally, the results show a high correlation (0.96) between the NPP and GP. Moderate associations were found between the GP and W100s and the W100s and NSP within six generations (P_1, P_2, F_1, F_2, BC_1, and BC_2) derived from crosses between LP 97-28 and IPR-Uirapuru [72].

9. Final considerations

This chapter described that the leading producers of chickpea worldwide are Nigeria, Niger, and Brazil, and this crop has a large influence on economy of these countries. However, the drought represents a problem frequently observed in areas with agricultural potential, with negative repercussion on production components and consequent decrease of the yield in this culture. Breeding programs aiming tolerance to drought using selection strategies linked to genetic, biochemical, physiological, nutritional, and agronomic characteristics can help increase the yield and reduce losses promoted by the drought. In addition, flower structure and hybridization technique used in Núcleo de Pesquisa Vegetal Básica e Aplicada (NPVBA/UFRA) were presented, as well as populations evaluated and plant management were explained in detail. This chapter described the results obtained in other breeding programs aimed at drought tolerance and also explained the potential uses to increase the crop yield.

Acknowledgements

This chapter had financial support from Fundação Amazônia Paraense de Amparo à Pesquisa (FAPESPA/Brazil), Universidade Federal Rural da Amazônia (UFRA/Brazil), and Conselho Nacional de Pesquisa (CNPq/Brazil) to Lobato AKS.

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