

Research Application Summary

Effect of heats stress on the germination of thirteen cowpea (*Vigna unguiculata* L. Walp) released varieties in West Africa

Agbahoungba, S.,* Assogbadjo, A.E. & Sinsin, B.

Laboratory of Applied Ecology (LEA), Faculty of Agronomic Sciences, University of Abomey-Calavi, 01 BP:526, Cotonou, Benin

Corresponding author: agbasympho@gmail.com

Abstract

Climate change presents scenarios of extreme temperature variations that will limit agricultural productivity further when the need for efficient production systems cannot be over emphasized. Aside breeding for tolerance, the development of new varieties of crops such as Cowpea, it is important to evaluate the temperature tolerance of new varieties prior to their release and commercialization. Therefore, in this study we evaluated the effect of the high temperatures on germination of 13 newly developed cowpea varieties resistant to major insect's pest and drought under laboratory conditions. The 13 cowpea varieties were evaluated for germination at 10°C, 20°C, 30°C, 35°C and 40°C. There was no germination for all the varieties at the extremes of 10°C and 40°C. However, at 20°C high germination percentage was recorded on the varieties IT93K-452-1 (100%), IT97K-499-35 (100%), Komcalle (100%), Kumassi (93.33%), Tvu-1509 (100%) while at 30°C the high germination percentage was recorded on Agbloto (100%), Akounado (93.33%), FUAMPEA1 (96.67%), FUAMPEA2 (93.33%), IT97K-499-35 (100%), IT97K-556-6 (83.33%), Komcalle (100%), Sanzi (90%). The temperature 35°C only provided high (100%) germination percentage for Komcalle. The variety TVU-1509 had the highest speed of germination. The variety IT 98K-205-8, and TVU-1509 had the highest homogeneity at the different temperatures. The results provide useful information for breeders on which varieties to release in a target environment and in formulating efficient selection and breeding programs.

Keywords: Cowpea, germinability; mean germination time, coefficient of velocity of germination, synchrony

Résumé

Le changement climatique présente des scénarios de variations de température extrêmes qui limitent davantage la productivité agricole lorsque la nécessité de systèmes de production efficaces ne peut être soulignée. Mis à part l'amélioration végétale pour la tolérance, le développement de nouvelles variétés de cultures telles que le niébé, il est important d'évaluer la tolérance à la température des nouvelles variétés avant leur certification et commercialisation. Par conséquent, dans cette étude, nous avons évalué l'effet des températures élevées en matière de germination de 13 variétés de niébé récemment développées et résistantes aux principaux organismes nuisibles et à la sécheresse en conditions de laboratoire. Les 13 variétés de niébé ont été évaluées pour la germination à 10 ° C, 20 ° C, 30 ° C, 35 ° C et 40 ° C. La germination fut nulle pour toutes les variétés aux températures extrêmes de 10 ° C et 40 ° C. Cependant, à 20 ° C, un pourcentage élevé de germination a été

enregistré sur les variétés IT93K-452-1 (100%), IT97K-499-35 (100%), Komcalle (100%), TVU-1509 (100%) et Kumassi (93,33%), tandis qu'à 30 ° C, Agbloto (100 ° C), Akounado (93,33%), Fuampea1 (96,67%), Fuampea2 (93,33%), IT97K-499-35 (100%), IT97K-556-6 (83,33%), Komcalle (100%), Sanzi (90%) on enregistrer de forte germination. La température de 35 ° C ne permet qu'un pourcentage de germination élevé (100%) pour Komcalle. La variété TVU-1509 avait la plus haute vitesse de germination. La variété IT 98K-205-8 et TVU-1509 avaient la plus haute homogénéité aux différentes températures. Les résultats fournissent des informations utiles pour sélectionneurs pour la production de variétés dédiées à des environnements cibles et pour formuler des programmes de sélection efficaces.

Mots-clés: niébé, taux de germination; Temps moyen de germination, coefficient de vitesse de la germination, synchronisation

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] is the most important crop legume in Sub Saharan Africa. It is a principal and multipurpose food legume in many African countries including Benin and Ghana where tender leaves, fresh pods, and dry grains are consumed (Alemu *et al.*, 2016). Cowpea is a multipurpose crop grown for human consumption, animal feed and improvement of soil fertility (Kpoviessi *et al.*, 2019). In Benin, cowpea is the first grown and consumed legume. It plays a key role in the subsistence and livelihoods of smallholder farmers, especially women who are the most involved in cowpea value chains (processing and commercialization) (Sodedji *et al.*, 2019, Kpoviessi *et al.*, 2020). The nationwide average yield of cowpea is about 800 kg/ha (DPP/MAEP, 2012), which is still low as compared to potential yield of 3.0 t/ha for most of the improved varieties (Kpoviessi *et al.*, 2019).

The low productivity of cowpea is due to several factors including biotic and abiotic stresses, (Saboya *et al.*, 2013; Boukar *et al.*, 2016). To address such biotic stresses many varieties have been developed in West African Cowpea Consortium and cowpea breeding programmes and evaluated in most of West African countries for release. However, their tolerance to temperatures stress during germination have not yet been established.

The increase in the planet average temperature will provoke extreme climatic events, such as droughts and heavy rainfall (IPCC 2013; Barros *et al.*, 2020). Cowpea development occurs in a wide temperature range between 18 and 37°C (Vale *et al.*, 2017). However, a temperature of 25°C as optimal for seed germination has been reported by many scientists (Tribouillois *et al.*, 2016 ; Barros *et al.*, 2020). Seed germination may be affected by the predicted increases in temperature. In addition, the arid and semi-arid regions in West Africa including Niger, Mali, Burkina-Faso and Benin that are among the major cowpea growing countries have been reported to be the more vulnerable to climate change and extreme temperature events (Barros *et al.*, 2020). Thus, to mitigate these events which are becoming unavoidable in the current centenary, it is highly desirable to have vigorous seed germination and uniform seedling establishment in these regions (Parmoon *et al.*, 2015 ; Barros *et al.*, 2020). Thus, the objective of this study was to evaluate the variation in germination process under stress temperature of thirteen cowpea newly developed varieties in West Africa breeding programmes.

Materials and methods

The germplasm used in this study includes 13 cowpea varieties that are resistant to aphid, flower thrips, striga and were developed by IITA, SARI (Ghana), University of Agriculture, Makurdi (Nigeria) and INERA Burkina-Faso and two landraces from Benin, Agbloto resistant to aphid and striga and Akounado reported to be tolerant to drought by farmers. The list of cowpea varieties used in the study and their characteristics are presented in the Table 1 below.

Table 1. Description of cowpea varieties used in this study

No.	Varieties	Resistance status to pests and weeds	Origin
1	SARC 1-57-2	Resistant to aphid	SARI (Ghana)
2	Sanzi	Resistant to flower thrips	Landrace (Ghana)
3	KVX442-3-25SH	Resistant to Striga	INERA-Burkina Faso
4	KVX771-10G		
5	UAM09 1055-6 (FUAMPEA 1)	Resistant to Striga	University of Agriculture,
6	UAM09 1051-1 (FUAMPEA 2)		Makurdi Nigeria
7	IT97K-499-35	Resistant to Striga	
8	IT97K-556-6	Resistant to aphid	IITA
9	Tvu-1509	Resistant to flower thrips	
10	IT 98K-205-8	Drought tolerant	
11	IT99K-573-1		
12	Agbloto	Resistant to aphid and striga	Benin landraces
13	Akounado	Drought tolerant	

Methods

The experiment was carried out in a completely randomized design, with three replications of 30 seeds, in a 13×5 factorial scheme, with thirteen cowpea varieties at the West African Centre for Crop Improvement, University of Ghana. The germination tests were conducted in petri dishes aligned in four different incubators. The incubators were set at five different temperatures: 10°C, 20°C, 30°C, 35°C and 40°C. All incubators were maintained at 100% ± 1 relative humidity, in darkness for eight days. Three replications of thirty seeds of each variety were randomly assigned to petri dishes.

The number of germinated seeds was counted daily (not cumulative counts) for eight days. Germinability (G), represented by the percentage of germination in the experimental conditions (Labouriau 1983), germination time (MGT) (Labouriau 1970), coefficient of variation of the germination time (CVGT) (Ranal and Santana 2006), germination rate (MR) (Labouriau 1970), uncertainty of germination (U) (Labouriau and Valadares 1976), synchronization index of germination (Z) (Ranal and Santana 2006 adapted from Primack 1980) and coefficient of velocity of germination (CVG) were used to describe the germination process.

Data were subjected to the analysis of variance in R software version 3.6.0 package (RCoreTeam

2019). The means of the different variables on the 13 cowpea varieties were separated using Fisher protected Least Significant Different test at 5% level of significance. The trends in the germinability of the varieties under the different temperatures have been plotted.

Results and discussion

The results revealed that there were significant differences among the 13 cowpea varieties for all the parameters (Table 2). The different temperatures caused significant effects among the varieties for all the germination parameters except for the coefficient of variation of germination time where no temperature effect was recorded. The interaction effects between varieties and temperatures were significant only for the germinability and the uncertainty of the germination process.

The trends in the germination percentage of the different varieties under the test temperatures revealed that for the temperatures 10°C and 40°C, no seed germination was recorded for all of the 13 varieties tested. The 20°C, 30°C and 35°C were able to break the dormancy of the seeds and differences were observed among the different varieties. At 20°C, the peak of germinated seeds was recorded on the varieties IT93K-452-1 (100%), IT97K-499-35 (100%), Komcalle (100%), Kumassi (93.33%), Tvu-1509 (100%) (Table 2). While the peak of germination at 30°C was recorded on the varieties Agbloto (100%), Akounado (93.33%), FUAMPEA1 (96.67%), FUAMPEA2 (93.33%), IT97K-499-35 (100%), IT97K-556-6 (83.33%), Komcalle (100%), Sanzi (90%) (Table 2). The temperature 35°C only provided high (100%) germination percentage for Komcalle. Apart from the varieties FUAMPEA 1 (96.67%), IT98K-205-8 (43.33%), IT93K-452-1 (96.67%) and Komcalle (100%) that showed constant germination percentage and the varieties Kumassi, Nafi and TVU-1509 that presented an increase in the germination percentage from 30°C to 35°C, the remaining varieties showed a decrease in the percentage of germinated seeds under the same temperatures.

The significant interaction effects between varieties and temperatures observed for the germinability (percentage of germination) and the uncertainty of the germination process indicated that varieties did not germinate the same rate at the different temperature. The temperatures 20°C, 30°C, and 35°C caused significant differences among the cowpea varieties indicating that these range of temperatures (20-35°C) created favourable conditions to the seeds to germinate through the increased production of enzymes required for the genetic processes as reported by Miransari and Smith (2014). Favourable temperatures from 20 to 35°C on cowpea cultivars (Acauã, Carijó, Guariba, Gurguéia, Itaim, Juruá, Pajeú, Potengi, Pujante, Rouxinol, Tapahium, and Tumucumaque) were reported by Barros *et al.* (2020) in Brazilia.

High germination percentage observed at 20°C for the varieties IT93K-452-1 (100%), IT97K-499-35 (100%), Komcalle (100%), Kumassi (93.33%), Tvu-1509 (100%) and at 30°C for varieties Agbloto (100%), Akounado (93.33%), FUAMPEA1 (96.67%), FUAMPEA2 (93.33%), IT97K-499-35 (100%), IT97K-556-6 (83.33%), Komcalle (100%), Sanzi (90%) are in agreement with the findings of Barrios *et al.* (2020) who reported that the temperature of 20°C provided germination above 96%, and the temperature range between 30 - 35°C favoured the germination speed index, average time, and seed germination speed. In fact, at 20, 25, 30, 35°C nitrogen

Table 2. Means of the germination parameters among the thirteen cowpea under the different temperatures

parameters varie.	Temp.	Varieties													L.S.D temp.	L.S.D	
		Agbloto	Akounado	FUAMPEA 1	FUAMPEA 2	IT 98K	IT93K	IT97K -205-8	IT97K -452-1	Komcallé -499-35	KUMASSI -556-6	Nafi	Sanzi	Tvu-1509			
G	10°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	20°C	96.67	53.33	90	83.33	16.67	100	100	76.67	100	93.33	86.67	83.33	100	4.84	10.08	
	30°C	100	93.33	96.67	93.33	43.33	96.67	100	83.33	100	86.67	90	90	86.67	4.84	10.08	
	35°C	83.33	73.33	96.67	90	43.33	96.67	93.33	60	100	93.33	93.33	86.67	93.33	4.84	10.08	
	40°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CVG	10°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20°C	51.9	35	38.5	44	35	61.8	53.8	31.8	48.9	57.5	43	42.1	72.4	5.3	11.03	
	30°C	76.6	54.8	67.9	72.3	51.9	76.5	76.4	63.4	88.9	74.6	60.7	67.5	88.6	5.3	11.03	
	35°C	59.4	51.6	62.4	61.1	50	60.5	68.9	40.5	72.4	55.1	65	67	92.3	5.3	11.03	
	40°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CVGT	10°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20°C	22.8	22.4	20.8	35.3	19.5	35.3	49.7	26.4	28	36.7	25.9	45.1	34.8	5.63	11.72	
	30°C	29.1	21.2	38.7	39.4	8.3	36.3	34.2	29.2	20.5	36.7	37.4	33.1	22.6	5.63	11.72	
	35°C	20.4	32.1	42.1	45.4	18	22.3	43.8	24.1	39.1	22.2	24.3	37.2	12.4	5.63	11.72	
	40°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Z	10°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20°C	0.63	0.43	0.53	0.39	0.34	0.47	0.36	0.43	0.47	0.38	0.44	0.17	0.53	0.08	0.17	
	30°C	0.70	0.64	0.45	0.48	0.87	0.54	0.59	0.58	0.82	0.51	0.46	0.51	0.76	0.08	0.17	
	35°C	0.70	0.38	0.40	0.58	0.60	0.66	0.43	0.48	0.53	0.67	0.65	0.44	0.84	0.08	0.17	
	40°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MT	10°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20°C	1.94	2.92	2.66	2.34	3.11	1.73	1.87	3.33	2.17	1.76	2.35	2.38	1.40	0.18	0.37	
	30°C	1.37	1.83	1.49	1.40	1.93	1.32	1.33	1.61	1.17	1.35	1.67	1.51	1.14	0.18	0.37	

	35°C	1.73	1.97	1.62	1.85	2.06	1.69	1.46	2.54	1.40	1.82	1.74	1.51	1.10	0.18	0.37
	40°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MR	10°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20°C	0.52	0.35	0.39	0.44	0.35	0.62	0.54	0.32	0.49	0.58	0.43	0.42	0.72	0.05	0.11
	30°C	0.77	0.55	0.68	0.72	0.52	0.77	0.76	0.63	0.89	0.75	0.61	0.68	0.89	0.05	0.11
	35°C	0.59	0.52	0.62	0.61	0.50	0.61	0.69	0.41	0.72	0.55	0.65	0.67	0.92	0.05	0.11
	40°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
U	10°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20°C	0.64	0.75	0.96	0.73	0.48	1.05	0.92	0.68	1.04	0.99	1.15	1.93	0.89	0.15	0.31
	30°C	0.64	0.62	1.07	1.02	0.24	0.87	0.81	0.80	0.33	0.90	1.12	0.90	0.46	0.15	0.31
	35°C	0.61	1.24	1.12	0.58	0.64	0.63	1.17	0.99	0.95	0.67	0.63	1.07	0.29	0.15	0.31
	40°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

G= Germinability; MT= Mean germination time; CVGT = Coefficient of variation of the germination time; MR =Mean germination rate; U= Uncertainty of the germination process; Z= Synchrony of germination; CVG= Coefficient of velocity of germination

compounds, including nitrous oxide enhanced seed germination through enhancing amylase activities (Zhang *et al.*, 2005; Hu *et al.*, 2007; Zheng *et al.*, 2009). However, no germination was recorded for all of the varieties at 10°C and 40°C indicated that these temperatures were out of the optimal germination temperatures of the varieties used in this study. This contrasts the findings of Shahidul *et al.* (2006) where high germination (>60%) in the cowpea cultivars CBE, PPH, TC-40, E, BBPH and TA were reported at low temperature (10°C). These differences between studies could be due to the differences in the genetics of varieties under test in the two studies. However, it has been widely reported that temperatures below the optimum result in a delay of seed germination due to reduced respiration-related enzyme activity and cellular metabolism (Taiz *et al.*, 2017). Each variety has a minimum temperature and a limit temperature below and above which germination does not occur (Motsa *et al.*, 2015) as is the case in this study for 10 and 40°C.

The decrease in the percentage of germinated seeds from 30 to 35°C for the cowpea varieties Agboloto, Akounado, FUMPEAL, IT97K-499-35, IT97K-556-6 and Sanzi could be explained by the fact that increasing temperature (5°C) reduced the percentage of seed germination by causing thermal stress, leading to inhibition of germination, and may cause thermal dormancy, affecting seed viability as reported by Bewley *et al.* (2013); Barrios *et al.* (2020). Indeed, the synthesis of plant hormones including abscisic acid and ethylene which control seed germination is mainly affected by temperature variation through the regulation of the enzymatic activities by promoting or inhibiting their synthesis (Gao-Takai *et al.*, 2019). This occurs because, elevated temperatures increase the levels of abscisic acid and promote the inhibition of genes responsible for the synthesis of gibberellin, an essential hormone for activation of the germinative process (Miransari *et al.*, 2014). In addition, high temperature can inhibit the synthesis of ethylene, another hormone responsible for seed germination. However, this is verified for some varieties including FUMPEA 1, IT98K-205-8, IT93K-452-1, and Komcalle,

that showed constant germination percentage when the temperature increases of 5°C and for the varieties Kumassi, Nafi and TVU1509 that presented an increase in the germination percentage when the increases of 5°C.

Conclusion

This study found that high germination percentage at 20°C was observed for the varieties IT93K-452-1, IT97K-499-35, Komcalle, Kumassi, Tvu-1509 and at 30°C for varieties Agbloto, Akounado, FUAMPEA1, FUAMPEA2, IT97K-499-35, IT97K-556-6, Komcalle and Sanzi. The variety TVU-1509 had the higher speed of germination at 35°C and the varieties IT 98K-205-8, and TVU-1509 presented the highest homogeneity at the different temperatures. The results provide useful information on cowpea seed germination at 20°C, 30°C and 35°C which will be useful for plant breeder and growers. Furthermore, selecting varieties/genotypes that have good germination under high or low temperatures could help extend the length of the production season.

Acknowledgments

This study was financed by UNESCO-TWAS through the IsDB-TWAS Postdoctoral Fellowship programme grant No 02/2020/Vendor no:506980. This paper is a contribution to the Seventh Africa Higher Education Week and RUFORUM Triennial Conference held 6-10 December 2021 in Cotonou, Benin.

References

- Alemu, M., Asfaw, Z., Woldu, Z., Fenta, B.A. and Medvecky, B. 2016. Cowpea (*Vigna unguiculata* (L.) Walp.) (Fabaceae) landrace diversity in northern Ethiopia. *International Journal of Biodiversity and Conservation* 8 (11): 297–309. <https://doi.org/10.5897/IJBC2016.0946>.
- Barros, J.R.A., Angelotti, F., Santos, J.O., Silva, R.M., Dantas, B.F. and Melo, N.F. 2020. Optimal temperature for germination and seedling development of cowpea seeds. *Revista Colombiana de Ciencias Hortícolas* 14 (2): 1-19. Doi:<https://doi.org/10.17584/rcch.2020v14i2.10339>.
- Bewley, J.D., Bradford, K. J., Hilhorst, H.W.M. and Nonogaki, H. 2013. Synthesis of Storage Reserves. Physiology of Development, Germination and Dormancy. 3rd Edition. *Seeds* 85–131. doi:10.1007/978-1-4614-4693-4_3
- Boukar, O., Fatokun, C.A., Huynh, B. and Roberts, P.A. 2016. Genomic Tools in Cowpea Breeding Programs : Status and Perspectives. *Frontiers in Plant Science* 7 (757): 1–13. doi:10.3389/fpls.2016.00757.
- DPP/MAEP (Ministère d’Agriculture d’Elevage et de la Pêche). 2012. Indice des Prix à la Production des Produits agricoles. <http://www.insae-bj.org/indice-prix-agricoles.html>.
- Gao-Takai, M., Ikegami, A.K., Matsuda, K., Shindo, H., Ueae, S. and Oyaizu, M. 2019. A low temperature promotes anthocyanin biosynthesis but does not accelerate endogenous abscisic acid accumulation in red-skinned grapes. *Plant Science* 283: 165–176.
- Hu K.D., Hu, L.Y., Li, Y.H., Zhang, F.Q. and Zhang, H. 2007. Protective roles of nitric oxide on germination and antioxidant metabolism in wheat seeds under copper stress. *Plant Growth Regulation* 53: 173–183.
- Intergovernmental Panel on Climate Change (IPCC). 2013. Mudanças climáticas 2013: The Physical Science Basis. Contribuição do Grupo de Trabalho I para o Quinto Relatório de

- Avaliação do Painel Intergovernamental sobre Mudanças Climáticas [Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V. and Midgley, P.M. (Eds.)]. Cambridge University Press, Cambridge, UK; New York, NY.
- Kpoviessi, A.D., Datinon, B., Agbahoungba, S., Agoyi, E.E., Chougourou, D.C., Sodedji, F.K.A. and Assogbadjo, A. E. 2020. Source of resistance among cowpea accessions to bruchid, *Callosobruchus maculatus* F. Coleoptera: chrysomelidae, in Benin. *African Crop Science Journal* 28 (1): 49–65, <https://doi.org/10.4314/acsj.v28i1.5>.
- Kpoviessi, A.D., Agbahoungba, S., Agoyi, E.E., Chougourou, D.C. and Assogbadjo, A.E. 2019. Resistance of cowpea-to-cowpea bruchid (*Callosobruchus maculatus* Fab.): Knowledge level on the genetic advances. *Journal of Plant Breeding and Crop Science* 11(8): 185-195. <https://doi.org/10.5897/JPBCS2019.0818>.
- Labouriau, L.G. 1970. On the physiology of seed germination in *Vicia graminea* Sm. *I. Anais da Academia Brasileira de Ciências* 42:235-262.
- Labouriau, L.G. 1983a. A germinação das sementes. Organização dos Estados Americanos. Programa Regional de Desenvolvimento Científico e Tecnológico. *Série de Biologia. Monografia* 24.
- Labouriau, L.G., and M.E.B Valadares. 1976. On the germination of seeds of *Calotropis procera* (Ait.) Ait. *f. Anais da Academia Brasileira de Ciências* 48: 263-284.
- Miransari, M. and D.L. Smith. 2014. Review on Plant hormones and seed germination. *Environmental and Experimental Botany* 99: 110–121.
- Motsa, M.M., Slabbert, M.M., Averbeke, W. and Morey, L. 2015. Effect of light and temperature on seed germination of selected African leafy vegetables. *Southern African Journal of Botany* 99 (1): 29-35. Doi: <https://doi.org/10.1016/j.sajb.2015.03.185>.
- Parmoon, G., Moosavi, S.A., Akbari, H. and Ebadi, A. 2015. Quantifying cardinal temperatures and thermal time required for germination of *Silybum marianum* seed. *Crop Journal* 3 (1): 145-151. Doi: <https://doi.org/10.1016/j.cj.2014.11.003>.
- Primack, R.B. 1980. Variation in the phenology of natural populations of montane shrubs in New Zealand. *Journal of Ecology* 68: 849-862. <https://doi.org/10.2307/2259460>.
- RCoreTeam. 2019. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL. <http://www.R-project.org/> R version 4.5.0.
- Saboya, R.C.C., Borges, P.R.S., Saboya, L.M.F., dos R. Monteiro, F.P., Souza, A.E.A. and Santo, A.F. 2013. Response of cowpea to inoculation with nitrogen-fixing strains in Gurupi-Tocantins State. *Journal of Bioscience and Bioengineering* 4 (1): 40-48. Doi: <https://doi.org/10.20873/jbb.uft.cemaf.v4n1.saboya>.
- Shahidul, I., Rafaela, C.C. and James, O.G. 2006. Screening for tolerance of stress temperature during germination of twenty-five cowpea (*Vigna unguiculata* L. Walp) cultivars. *Journal of Food, Agriculture and Environment* 4 (2): 191-195.
- Sodedji, K.A.F., Agbahoungba, S., Nguetta, S.P.A., Agoyi, E.E., Ayenan, M.A.T., Sossou, S.H., Kouassi, B.A., Mamadou, C., Assogbadjo, A.E. and Kone, D. 2019. Cowpea resistance to legume pod borer (*Maruca vitrata* Fabricius): genetic advances, challenges and future prospects. *Journal of Crop Improvement* 34 (2): 238-267. <https://doi.org/10.1080/15427528.2019.1680471>.
- Tribouillois, H., Dürr, C., Demilly, D., Wagner, M.H. and Justes, E. 2016. Determination of Germination response to temperature and water potential for a wide range of cover crop species and related functional groups. *PLoS ONE* 11 (8): e0161185. <https://doi.org/10.1371/journal.pone.0161185>.

- Vale, J.C., Bertini, C. and Borém A. 2017. Feijão-caupi: do plantio à colheita. Editora UFV, Viçosa, Brazil.
- Zhang, H., W.B. Shen, W. Zhang, and L.L. Xu. 2005. A rapid response of-α-amylase to nitricoxide but not gibberellin in wheat seeds during the early stage of germination. *Planta* 220: 708–716.
- Zheng, C., Jiang, D., Liu, F., Dai, T., Liu, W., Jing, Q. and Cao, W. 2009. Exogenous nitric oxide improves seed germination in wheat against mitochondrial oxidative damage induced by high salinity. *Environmental and Experimental Botany* 67: 222–227.