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VARIABILITY IN SEED GERMINATION CHARACTERISTICS OF *CAPSICUM ANNUUM* L. AND *CAPSICUM FRUTESCENS* L.

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Abstract

Seed germination, a phylogenetically conserved trait, is crucial for seedling establishment and survival in nature and can be used to establish phylogenetic relatedness among plant species. The current study evaluated seed germination characteristics of varieties of two closely related cultivated *Capsicum* species in West Africa in response to light, temperature and sowing depth under controlled environment. The varieties and species used in this study are *C. annuum* var. *grossum*, *C. annuum* var. *abbreviatum*, *C. annuum* var. *accuminatum* and *C .frutescens* var. *baccatum*. The results indicated that the varieties showed similar and overlapping germination requirements although considerable differences were also observed. However, the differences observed among the varieties do not provide enough evidence to conceive that the collections are two separate species but rather suggest that they are phylogenetically related. Additional comparative studies using DNA molecular profiling are needed to confirm the relatedness and variability in these species.

Key words: Phylogenetic, *Capsicum*, Seed germination, Variability, Species.

Introduction

The genus *Capsicum* commonly known as chilli or hot pepper belongs to the family Solanaceae. Approximately, this genus consists of five domesticated species and twenty-two (22) wild species. The domesticated ones are *C. annuum* L., *C. Chinenses* Jacqs., *C. frutescens* L., *C. pubescens* R. and *C. baccatum* L. (Bosland & Votava, 2000).

In West Africa, the genus is represented by two cultivated species namely *C. frutescens* and *C. annuum* with different varieties. Unfortunately, the taxonomic identity of these species has been difficult as they have overlapping morphological characters, which has led to unresolved species identification. A persistent question in their taxonomy is whether these cultivated species are two different species or botanical varieties of the same species. According to (Bosland & Votava, 2000), "species is a population or series of populations within which free gene flow occurs under natural conditions, with fertile and healthy progeny produced by interbreeding within the species whereas a botanical variety is a distinct morphological subgroup within a species".

C. annuum and *C. frutescens* were first distinguished as the only two species in the genus *Capsicum* by Linneaus in 1825. This distinction was based mainly on whether the form was annual (annuum) or perennial (frutescens). However, in the study of South American pepper, Bukasow (1930), did not accept Linneaus view and considered the annual form to be nothing more than a variety of *C. frutescens*. Today, these two species are currently included among the five domesticated species in the genus *Capsicum*.

In West Africa, the different cultivated species of *Capsicum* are usually distinguished morphologically by combinations of flower and fruit characteristics and hybridization studies (Pickersill, 1991). The species exhibit considerable morphological variations in flower and fruit traits. There are varieties that possess one or more diagnostic morphological characters from one species and the rest of the characters from the other species thus, creating difficulties in species assignment. Also, there is free flow of genes among varieties of the cultivated *Capsicum* species in West Africa through intraspecific hybridization resulting in a baffling range of types. Therefore, the question of whether *Capsicum annuum* and *Capsicum frutescens* are distinct species still persists. The knowledge of relatedness among species and plant evolution could be constrained by dearth of information on the reproductive studies of plant species (Grant, 1981; De Queiroz, 2007; Stuessy, 2009).

Seed germination is a vital stage in the life cycle of flowering plants; which is determined by different environmental factors and by a large number of genes (Koornneef *et al*., 2002; Finch-Savage & Leubner-Metzger, 2006; Bewley *et al*., 2013). Like any other trait, seed germination in plant species has been found to be a phylogenetically conserved trait and very close species are likely to show similar values for a particular character than species that are distantly related (Norden *et al*., 2009). The variability in seed germination may be used to establish phylogenetic relationship among the varieties of the cultivated *Capsicum* species.

The study therefore aimed to determine whether the varieties of the cultivated *Capsicum* species namely *C. annuum* var. *grossum*, *C. annuum* var. *accuminatum*, *C. annuum* var. *abbreviatum* and *C. frutescens* var. *baccatum* respond similarly or show differences in seed germination characteristics and to check if the variation could be used to establish relatedness among and within these species.

Materials and Methods

Collection of plant materials: Nigeria is a major producer and consumer of the cultivated varieties of *Capsicum* species in West Africa, therefore, mature fruits of the four varieties of *Capsicum annuum* and *Capsicum frutescens* were obtained from markets in major geographical zones in Nigeria. Seeds in the fruits were first removed, sun-dried and stored at room temperature of about 15-25°C in paper bags and were later used for the germination experiments. The taxonomic labels of the *Capsicum* varieties are *Capsicum annuum* var. *abbreviatum* - rodo, Capsicum *annuum* var.

acuminatum –sombo, *Capsicum annuum* var. *grossum* -tatase and *Capsicum frutescens* var. *baccatum* -wewe. The voucher specimens of each variety was collected and deposited at the University of Ilorin's herbarium.

Viability test*:* Seed viability was tested using tetrazolium technique to ensure that the seeds were of high quality and viable (Grabe, 1970). For each seed lot, three replicates each consisting of 50 seeds were used. Following the procedures of Peters (2000) for the family Solanaceae, "the seeds were soaked in water for 24 hours, laterally cut through the entire depth without destroying the embryo. These were then put in 0.1% solution of 2, 3, 5-triphenyltetrazolium chloride (TTZ) in the dark at 25°C over a period of 16 hours. The TTZ solution was decanted and the seeds rinsed thoroughly in water, and were transferred into 10 ml ethyl alcohol (95%) to allow for the embryo to be easily observed. Metabolically active (viable) seeds embryos were stained red.

Seed weight determination: For each variety, the average weight of 100 air-dried seeds, were calculated from five replicates to determine the seed weight.

Effects of light: The effects of light (continuous light, alternating light/dark and continuous darkness) were verified in a controlled environment system. Three replicates of 25 seeds of each variety of *Capsicum* were placed in a Petri dish lined with double Whatman No1. filter paper and moistened with 3 ml of distilled water. Continuous white light was supplied from four florescent tubes and maintained at 1.5 m above the seeds. The dark treatments were kept completely dark all through the period of the experiment by wrapping the Petri dishes with two layers of aluminum foil and daily observations were made in a dark room illuminated with green light. All the seeds were checked daily for germination for 14 days.

Effect of temperature: This was examined by putting the Petri dishes containing the seeds in incubators set at 5, 10, 15, 20, 25, 30, 35 and 40°C respectively. These were daily observed for 14 days. Distilled water was added when required. Germination was scored based on radicle protrusion (2mm long).

Effect of sowing depth: This was determined by sowing 10 seeds in seedling germination trays filed with top loamy soil at depths of 0 (soil surface), 1, 2, 3, 4 and 5 cm with three replicates for each variety. The trays were sub-irrigated to minimize soil crusting and compacting. Each tray was watered daily and germination count was taken for 14 days.

Statistical analysis: For all treatments, two germination variables were considered: final seed germination cumulative percentage (germination percentage) and half germination time (T50) (germination rate). A two way analysis of variance (ANOVA) was performed to compare treatments within and among the varieties. Using MINITAB 17 statistical package, mean separation was done using the Fisher's LSD where the data showed significant ($p<0.05$) difference.

Results

Viability and seed weight: The viability of the seeds of the *Capsicum* species studied were high, ranging from 90% in rodo to 95.33% in tatase. The highest viability was recorded for tatase (95.33%) followed by var. sombo (94%), wewe (92.66%) and rodo (90%). Across the varieties, the mean weight of 100 seeds ranged from 0.35 ± 0.004g to 0.47 ± 0.003g. Seed weight did not vary significantly among the varieties. However, sombo had the highest weight (0.47 ± 0.003g) followed by tatase (0.43 ± 0.006g) and rodo (0.38 ± 0.002g). The lightest seeds were those of wewe (0.35 ± 0.004g).

Effect of temperature on germination*:* There were significant differences in germination among and within the variety in response to temperature treatment. There was no germination at low temperatures of 5°C and 10°C and at higher temperatures of 35°C and 40°C (Fig. 1). In all the varieties studied, there was increase in germination with temperature from 15°C up to 30°C. Generally, the optimum T_{50} was observed at 25°C within and among the *Capsicum* species (Fig. 1). At the optimum temperature, sombo had the highest percentage germination (98.67%) followed by rodo (96%), and tatase (93%) while the least germination was recorded in wewe (80%). Nonetheless, there was no significant difference in percentage germination in all varieties at 25°C and 30°C. In this treatment, mean T_{50} among the four varieties of *Capsicum* species at the optimum temperature was 6.48 days and it ranged from 4.60 days to 8.33days (Fig. 2).

Effect of light on germination*:* All the varieties of the *Capsicum* species did not differ significantly in percentage germination in the continuous light and alternating light/dark treatments (Fig. 3). However, rodo, differs significantly from sombo and tatase in the continuous darkness treatment, but there was no significant difference among sombo, tatase and wewe in the continuous darkness treatment (Fig. 3). The best condition for seed germination in this experiment was the continuous darkness in sombo (100%), tatase (89.33%) and wewe (92%) whereas the highest germination percentage was recorded in the alternating light and darkness in rodo (90.67%). Germination rate varied significantly within and among the species in continuous light and alternating light and darkness. Generally, the earliest T_{50} occurred in the continuous darkness in all the varieties and it ranged from 4.3 to 8 days whereas the most delayed (6 to 8.33 days) occurred in the continuous light treatment (Fig. 4).

Effect of sowing depth on germination: In germination percentage and germination rates, no significant difference was observed within and among the species at the different sowing depths. However, Var. wewe differs significantly from rodo, sombo and tatase at a sowing depth of 1cm (Fig. 5). In general, germination rate observed in this study diminished progressively with depth. The maximum germination percentage and emergence for all varieties occurred at a sowing depth of 1cm followed by 2 cm and 3 cm (Fig. 5). No germination occurred at 4 cm and only rodo germinated at 5 cm below the soil. In this treatment, average T_{50} at the optimum sowing depth (1cm) among the varieties was 10.66 days with a range of 9.66 days to 11.33 days (Fig. 6).

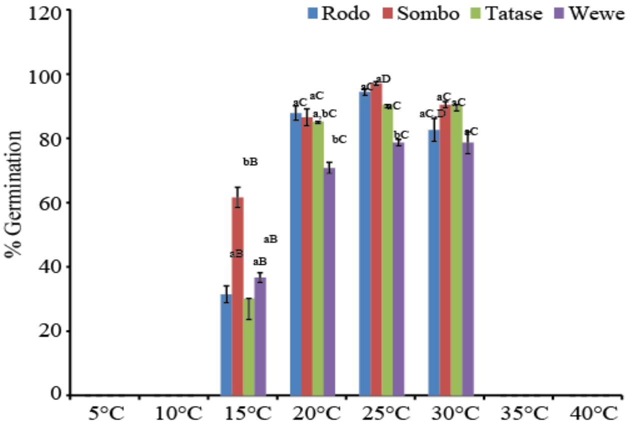

Fig. 1. Effect of temperature on the seed germination of varieties of *Capsicum* species. Values are mean ± standard deviation. Different lower case within group of bars indicates significant difference ($p<0.05$) among the varieties while different upper case within bars of the same colors indicates significant difference within a variety.

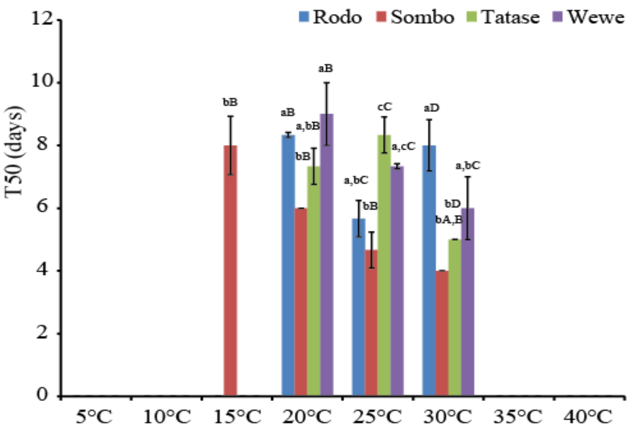

Fig. 2. Effect of temperature on the germination rate (T_{50}) of varieties of *Capsicum* species. Values are mean ± standard deviation. Different lower case within group of bars indicates significant difference ($p<0.05$) among the varieties while different upper case within bars of the same colors indicates significant difference within a variety.

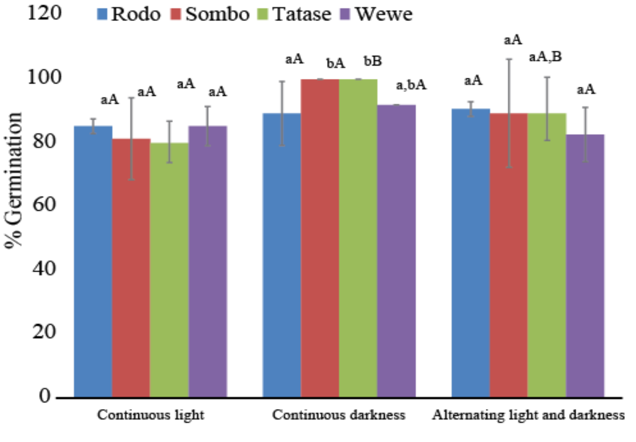

Fig. 3. Effect of light on the seed germination of varieties of *Capsicum* species. Values are mean ± standard deviation. Different lower case within group of bars indicates significant difference ($p<0.05$) among the varieties while different upper case within bars of the same colors indicates significant difference within a variety.

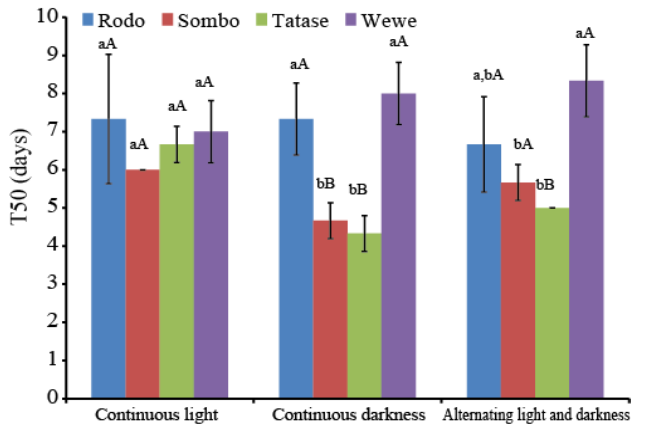

Fig. 4. Germination rates (T_{50}) of varieties of *Capsicum* species under light treatment. Values are mean ± standard deviation. Different lower case within group of bars indicates significant difference ($p<0.05$) among the varieties while different upper case within bars of the same colors indicates significant difference within a variety.

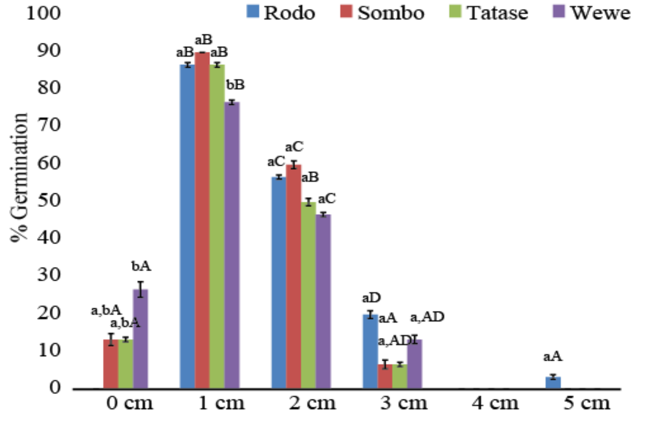

Fig. 5. Effect of sowing depth on the seed germination of varieties of *Capsicum* species. Values are mean ± standard deviation. Different lower case within group of bars indicates significant difference ($p<0.05$) among the varieties while different upper case within bars of the same colors indicates significant difference within a variety.

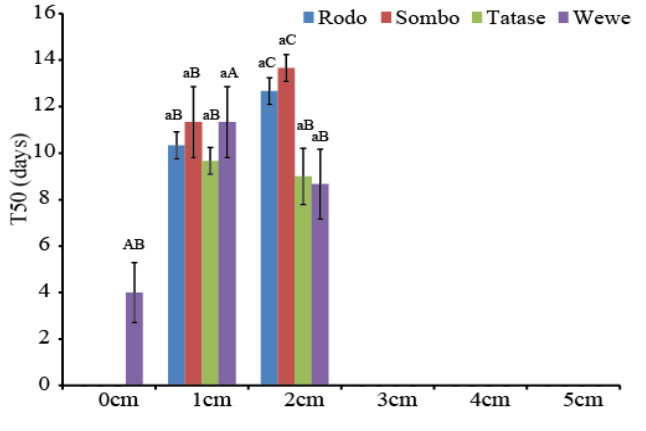

Fig. 6. Germination rate (T_{50}) of varieties of *Capsicum* species under varied sowing depth. Values are mean ± standard deviation. Different lower case within group of bars indicates significant difference ($p<0.05$) among the varieties while different upper case within bars of the same colors indicates significant difference within a variety.

Discussion

Generally, phenotypic variation is assumed to reveal the innate genotypic variation within and among plant species grown in the same conditions (Abdelbasit *el al*., 2014). Seed traits, including weight, germination requirement and germination rate are key components of plant life histories. They comprise co-evolved complexes for many plant species that may be associated with relatedness among many species (Aaron, 2001). In this study, the germination behavior of each variety of *Capsicum* species varied in response to light, temperature and sowing depth and this variation in germination behavior within and among the species may reveal the overriding effect of both genetic and environmental variation which may reflect true variation. Variations in seed germination characteristics among and within varieties of the same plant species have been reported for many plants (Dangasuk *et al*., 1997) and may be ascribed to genetic differences resulting from their adaptations to different environmental conditions and soil types (Ginwal *et al*., 2005; Elmagboul *et al*., 2014).

According to Moore (1985) and Leist & Kramer (2003), TTZ test has been reported to generally agree with seed germination tests, and has been determined for about 650 plant species. The high viability of the seeds of the four varieties of *Capsicum* species in this study showed that the seeds are viable.

The four varieties were not significantly different in seed weight. However, sombo had both the highest mean seed weight and germination percentage in all treatments. This high percentage germination may be because heavier seeds have larger embryos and more endosperm nutrients, resulting to increased germination percentage as reported by Lo´pez-Castan˜eda, (2006).

Seeds of the four varieties of *Capsicum* species showed considerable variation in germination in response to light treatment. The four varieties germinated above 70% in all the light treatments. According to Bosland & Votava (2000), there were no special requirements for light in pepper seed germination as fluorescent light sources in germination cabinets did not inhibit *Capsicum* seed germination; however, they did not promote germination either. Therefore, the absence or presence of light is not a factor in determining seed germination of *Capsicum* and this will allow germination to occur beneath the surface of the soil. This is in line with the results of this work.

Germination increased linearly with increasing temperature in rodo, sombo, tatase and wewe from 15°C up to 30°C but the germination percentage and germination rate varied between the varieties. According to Ginwal *et al*., (2005), this variation may be ascribed to genetic differences as a result of adaptation to different environmental conditions and soil types (Elmagboul *et al*., 2014). The result of the moderately high optimum temperature (25°C) recorded for germination suggests that high day time temperatures, which are known features of tropical and sub-tropical parts, are more suitable for the four varieties of the *Capsicum* species (Mosta *et al.*, 2015). Also, the low germination observed in the four varieties at lower temperature than 15°C and at higher temperatures (35°Cand 40°C) may be an indication that no or little germination will occur during mid-summer or winter and this could be a defense mechanism to prevent extreme seedling death (Chanyenga *et al.*, 2012). This corroborates the findings of Balkaya (2004) and Tokumadu *et al*., (1985) who reported poor rate of germination at very high and low temperatures in *Brassica* and *V. unguiculata* species respectively.

The germination percentage and germination rate did not vary in respect to sowing depth in rodo, sombo, tatase and wewe and germination decreased progressively with increasing sowing depths. In comparison to seeds sown at 1cm, there was low emergence in seeds that were planted on the soil surface. Poor seed germination on the soil surface may be caused by declined water availability and limited soil to seed contact (Clewis *et al.*, 2007). There has been several reports of low seed emergence in various crops and weed species due to increase in sowing depth (Lu *et al*., 2006; Norsworthy & Oliveira 2006; Vidal *et al*., 2007; Linda & Afolayan, 2015; Jeremi, 2018) and this could be as a result of little light transmission below a depth of 4 mm in the soil as reported by Benvenuti *et al.*, (2001) or not enough nutrients to sustain the emerging seedling.

Generally, the results from the study showed that the varieties and species share similar germination requirement in respect to light, temperature and sowing depth as wewe (*Capsicum frutescens* var. *baccatum*) did not clearly standout distinctively from rodo, sombo and tatase which are varieties of *Capsicum annuum*. This suggests that the four varieties may belong to one species (*Capsicum annuum*).

Conclusion

The study affirmed the hypothesis that phylogenetically close species show similar germination traits. The four varieties of *Capsicum* species responded similarly in their germination requirements and their germination takes place optimally at a range of 20-30°C, 1cm sowing depth under light and darkness. The differences in the seed germination performances within and among the varieties of the *Capsicum* species did not distinguish the two species as separate species. Additional comparative studies using DNA molecular profiling and other approaches are needed in the on-going effort to fully determine the relatedness or variability in these two species.

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