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## RESEARCH PAPER

# EFFECTS OF SOIL MOISTURE STRESS ON THE VEGETATIVE ANATOMY OF SIX COWPEA ACCESSIONS FROM THREE AGRO-ECOLOGICAL ZONES IN GHANA

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## ABSTRACT

*Climate change has been affecting the rainfall patterns that influence the soil moisture supply in most areas of the tropical and sub-tropical regions. Soil moisture has thus, become a problem to farmers and agronomists in these agro-ecological regions as a result of the deleterious effects that inadequate osmotic water has on the morphology and anatomy of growing plants. This study determined the effects of soil moisture stress on the tissues of the plants of six cowpea accessions. Six accessions of cowpea were collected from the three agro-ecological zones and planted in polythene bags containing 2 kg each of well mixed loamy soil. The plants were allowed to grow for three weeks before being subjected to two water stress treatments with a third being a control. Harvests were done through random sampling on three occasions. Tissues were obtained from the plants and prepared on slides for study. The mean values of all the parameters measured were subjected to statistical tests. There were significant reductions in the sizes of the cells and tissues subjected to the moisture stress regimes ($P<0.05$, $df=2$). Among the plants, there were non-uniformed variations at 0.05 confidence limit and $n=6$. Most of the cells plasmolysed under the stress treatment and resulting in the reduction of the tissue sizes. Exceptions to this trend of decreasing sizes of the tissues were found in the sizes of the cuticular cover on the surfaces of leaves and the frequency of the xylem vessels, where these features increased in sizes and frequency respectively under the water stress regimes. The accession from the Semi-deciduous ecological zone of Ghana performed better under the moisture stress regimes. The study identified accessions of cowpea that can grow better under inadequate soil moisture condition that is prevalent in the sub-tropical region of Africa, and lead to high yields in the production of the legume crop.*

**Keywords:** Anatomy, Accessions, Moisture stress, Plasmolysed, Ecological zone

## INTRODUCTION

Drought is an important environmental factor affecting plant growth in the tropics due to climate change (Abayomi and Abidoye 2009; Antwi-Agyei, *et al* 2017). Drought occurs frequently in arid and semi-arid regions and may be due to erratic start and early cessation of rainfall regimes (Shao *et al* 2009). This results in an inadequate water supply to plants and brings about soil moisture stresses on the plants.

Ghana is an agricultural country and most farmers engage in subsistence farming. The farmers however depend mostly on rainfall for their water supplies. Cowpea is one of the arable crops grown, and possesses a relatively high protein index (Lobato *et al* 2008). It is only second in importance to cereals as the source of food for humans in Africa. The plants of cowpeas are found to grow in the Semi-deciduous and the Savanna ecological zones in Ghana where annual rainfall is not excessive (Antwi-Agyei *et al* 2017).

The world demand for cowpeas as a protein supplement is on the high side (FAO 2016; Kraso-Wade *et al* 2006). There is the need therefore, to produce more cowpeas to meet the farming communities and the ever-increasing human population. But the production is being hindered by many factors; with soil moisture stresses being the most prevalent. Soil moisture stress creates internal pressure deficits and osmotic imbalances in the tissues of plants and these tend to reduce the sizes of the tissues; thus the growth and fruit yields of plants (Aakansha, *et al* 2019).

Plants have been found to develop adaptive mechanisms to forestall inhibitions to their developmental and reproductive growth stages that may be posed by moisture stress (Eung-Pill *et al* 2017; Ezin *et al* 2021). These mechanisms could be morphologic, physiologic or anatomic, and may be expressed innately by the inherent genes or as a temporal modification due to unfavorable environmental conditions (Eung-Pill, *et al* 2017).

Effects of water stresses on the tissues of plants gained popularity in the 1960s to the 70s though the subject is scarcely mentioned of late. Many researchers and authors have been looking at the effects of water stress on plants from the physiological point but not from the anatomy. It should; however, be noted that any stress that is imposed on plants through the absorptive process affects the tissues first before being manifested morphologically and on fruit yield (Prihar *et al* 2000; Mahajan *et al* 2020). When cells and tissues in plants plasmolyse, the plants shrink in size and result in stunted growth, and subsequent wilting (Costa *et al* 2008; Shao *et al* 2008). This study investigated the effects of water stress on the tissues of six accessions of cowpeas from three agro-ecological zones in Ghana. Cowpea production in Ghana is characterized by variable rainfall and inadequate soil moisture (Antwi-Agyei *et al* 2017; Hall 2004), and there is a need to identify accessions that could tolerate soil moisture deficits in order to improve the production of the legume crop.

## MATERIALS AND METHODS

### Experimental set-up

Six accessions of cowpeas, with two from each of the three agro-ecological zones in Ghana were acquired from PGRRC (Bunso), and labeled SD1, SD2 (Semi-deciduous), CS1, CS2 (Coastal Savanna) and SS1, SS2 (Sudan Savanna). The study was conducted in the Plant House of the Biology Department of the Kwame Nkrumah University of Science and Technology under controlled conditions. A viability test was performed on the seeds in the laboratory before they were planted in poly bags containing 2 kg of well mixed soil each. The plants were allowed to grow till the end of the 3rd week when they were arranged under three moisture stress regimes; 0% moisture stress (T0), 50% moisture stress (T1) and 83% moisture stress (T2). Each stress regime had 15 replicates of each accession because of the destructive nature of the sampling, and giving the total sample size was 270 plants. A 6X3 factorial experiment in a completely randomized design was thus employed with the six levels of accession (SD1, SD2, CS1, CS2, SS1, SS2), and three levels of moisture stress (0%, 50%, 83%).

With the water holding capacity of the soil of 42.21% in mind, the soil was stressed at 50% and 83% for the medium and severe stress regimes respectively. Watering was done on the same days with the variation in the amount of water supplied. The normal irrigation rate was 240 ml of water every three days (0% moisture stress). The medium stress regime was given 120 ml of water every three days (50% moisture stress) and the extreme stress protocol given was 40 ml of water every three days (83% moisture stress). The moisture stress doses applied are indicated by Table 1.

**Table 1. Moisture stress levels applied**

| Treatment code | Moisture regimes | Water supplied (mls/3days) | Moisture stress (%) |
|---|---|---|---|
| T0 | Normal irrigation | 240 | 0 |
| T1 | Medium stress | 120 | 50 |
| T2 | Extreme stress | 40 | 83 |

Five plants of each accession under the different water treatment protocols were harvested by random sampling at the end of the 3rd, 5th,and 7th weeks of the treatment period. The stress application was monitored periodically before each harvest by the relative soil moisture content method. Some environmental conditions were measured daily through the use of a thermo-hydrograph and a light meter. The experimental period lasted for seven weeks.

## Anatomical preparation

The plant parts investigated anatomically were that of the roots, stems and leaves. The roots were cut at a distance of 2.0 cm from the base of the stem and fixed in formol-aceto-alcohol (FAA). This length was taken because the root branches profusely after the 2.0 cm length in all the plants. Samples of the stems of the plants were taken at points of 1.0 cm distance above the first, second and third nodes of the harvested plants. This was to prevent the effects of branching on the vascular arrangements in the stems from affecting the investigation. For the leaves, the middle leaflets of the 2nd, 3rd,and 4th leaves from the base of the stem were chosen. A cut was made transversely across the leaflets at 1.0 cm from the trifoliate joint and a 2.0 cm piece of the leaflets that included the mid vein was taken. These preparations were made for each of the five plants harvested on each sampling occasion. The organs fixed in the F.A.A were subjected to the processes of permanent slide preparation; ie. dehydration, infiltration, embedding, microtomy, staining and mounting. The methods used for the preparation of the permanent slides were adopted from that used in earlier works by Arkoh (2010) and Abayomi and Abidoye (2009).

### Anatomical study

The prepared slides were studied under a light microscope with a scanning magnification of X40 and a detail study of X400. The variables measured for the stems and roots samples were diameters of epidermal, cortical, pith cells and xylem vessels, and the width of the cortex, pith region and vascular band/ bundles, and the frequency of xylem vessels. The variables measured for the leaves were diameters of the epidermal, palisade and spongy cells, widths of cuticle layers, vascular bundle, stomata guard cells, pores of the stomata, and frequency of stomata pores. The stomata study was performed immediately after harvesting of the plants to prevent distortions in the shapes of the stomata.

In the study, the stage of the microscope was manipulated to change the field of view of the slides under observation to enable the measurements to be taken from different orientations. The measurements were taken in microns (μ) for over 20 cells for each variable. The means were recorded and analyzed.

### Statistical analysis

The means of the data collected were subjected to SPSS software and subjected to analysis of variance (ANOVA) and where significant differences were observed, Duncan's Multiple Range Test (DMRT) was used to separate the means.

## RESULTS AND DISCUSSION

The results of the soil moisture content analysis (Table 1) showed decreases in the mass of the soil water with increases in moisture stress. The measured atmospheric conditions showed no wider variation within the three months of the study (September-November).The mean temperature was 26.73 $^{o}$C, the percentage relative humidity ranged between 64-69 %, and the mean light intensity was 12.05 Lux. A significant decrease in the mean soil moisture values observed in this work along the treatment protocols indicated a positive stress application (Table 2).

**Table 2. Variations in mean soil moisture content (%) on sampling occasions**

| Weeks of sampling | Moisture stress (%) | | |
|---|---|---|---|
| | 0 | 50 | 83 |
| 2$^{nd}$ | 23.75 ± 0.05 a | 15.13 ± 0.02 b | 9.67 ± 0.03 c |
| 4$^{th}$ | 9.10 ± 0.02 a | 5.73 ± 0.03 b | 2.30 ± 0.02 c |
| 6$^{th}$ | 3.70 ± 0.02 a | 1.01 ± 0.01 b | 0.13 ± 0.01 c |

*By the calculated confidence limit of 95%, the means and S.E values bearing the same letters along the row are not significantly different.

## Plant growth under soil moisture stress

Plants are known not to grow well in low soil moisture condition as was observed under this study. However, results from a study by Aakansha *et al* (2019) indicated that *Lactuca serriola* was able to survive and produce seeds under water severe soil moisture stress conditions as more than 6000 seeds were produced per plant in all low water holding capacity (WHC) treatments of their study. This could have a huge impact on agricultural systems as the species could survive under both low and high soil moisture conditions (Aakansha, *et al* 2019). Also plants like *Avena ludoviciana* have been indicated to survive and produce seeds at 40% WHC, showing the adaptation of the species to drier land conditions. They also, suggested that *A. ludoviciana* is likely to be robust under water stress conditions, and potentially increase crop yield (Mahajan, *et al* 2020).

## Anatomical variations in the roots and stems

Table 3 shows the measured anatomical parameters of the roots and stems of the plants of the different accessions under the water stress regimes. The accessions of cowpeas used were given experimental codes with SD1 and SD2 being two accessions from the Semi-deciduous ecological zone, CS1 and CS2 were for two accessions from the Coastal savanna, and SS1 and SS2 being accession from the Sudan Savanna zone in Ghana. The measured variables showed a reduction in sizes generally, under increasing water stress regimes. The diameters of cells and width of tissues reduced in sizes along the stress protocols with the highest reduction being seen in the pith region under the severe treatment regime.

The variations observed were found to be insignificant (P>0.05, df=2) for the epidermal cells under the water stress protocols but significant for the cortical and pith cells at P<0.05. Epidermal cells are thickened and flattened cells at the outer portion of plants and serves as protective tissue to the internal portion (Ashraf *et al* 2017; Boyer 1985). Most epidermal cells die at maturity and become strengthened, thus osmotic imbalances due to water stress have little effect on them (Boyer 1985; Costa *et al* 2008) as was observed in this study. The variations among the plants of the accessions were also not statistically significant P>0.05, df=5 and exhibited a low lower spread (CV(%) = 1.25%).

The cortical and pith tissues varied greatly in decreasing sizes along the water stress regimes (Table 3). These tissues are made up of parenchyma cells which are not thickened and could easily react to osmotic imbalances. The cells shrink in size under inadequate water availability which may lead to plasmolysis and wilting in severe situations (Eung-Pill *et al* 2017; Prihar *et al* 2010). There was a wider reduction in the cell sizes of the cortex tissue between the 50% and 83% regimes due to the presence of some form of thickening in cortical cells. Some cortical cells may develop chlorenchyma features upon stress imposition and the cells may have a little reduction in sizes under medium (50%) stress as was observed in this study. Under severe stress (83%), the cells tend to respond to the stress due to the degree of the stress imposition. However, the pith cells showed significant reductions (P<0.05) even under 50% stress because the cells are purely parenchymatous in nature without any thickening (Kowslowski 1972; Shao *et al* 2008). The CV (%) among the accessions indicated a smaller degree of variation of 0.50%. Gaps appeared between the cells of the pith under the 83% stress regime in all the accessions. It could be speculated that the middle lamella binding the pith cells weakened and the cells dissociated from one another, thus forming the gaps that appeared.

An exception to this trend of reductions in the sizes of the tissues was found in the vascular system of the plants. The frequency of xylem vessels increased along the treatment protocol with the vessels having a smaller lumen. The increase in the number of xylem vessels with smaller lumen was an adaptive method initiated by the meristematic cells of the cambium to help the plants withstand the drought condition; the imposed water stress (Boyer 1985).

Basic physics asserts that columnar objects with smaller radii need little pressure to enable water or fluid to rise upwards in the tube. Thus, the reduction in the sizes of the xylem vessels might have facilitated the flow of inadequate soil water absorbed by the roots of the plants up the stems to replace the water lost through transpiration (Hall 2004; Eung-Pill *et al* 2017).

Accessions from the Coastal and Sudan savanna zones could not stand the severe stress protocols till the end of the experimental period. Soil moisture deficits might have created a dilapidating effect on the accessions as a result of over-transpiration against available water uptake by the plants, thus causing a reduction in sizes and subsequent. Prihar *et al* (2010) realize reductions in the sizes of the plants under moisture deficit conditions and attributed the rate of biomass accumulation or deficiency to the amount of radiation intercepted by the plants, and that the developmental rate was influenced by the temperature experienced by the plants in an earlier study.

Table 3: Mean and S.D data on the root and stem measurements of the cowpeas studied under the different water treatments

| ACCESSIONS CODES | Moisture stress (%) | MEAN VALUES OF MEASURED PARAMETERS / X10µm | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Epidermal cells diameter | Cortical cells diameter | Width of cortex | Width of vascular bands | Diameter of xylem vessels | Frequency of xylem vessels | Sizes of pith cells |
| SD1 | 0 | 2.22± 0.02a | 3.35±0.03a | 22.50±0.04a | 26.18±0.02a | 8.24±0.01a | 10.33±0.03a | 10.46±0.02a |
| | 50 | 2.21± 0.02b | 3.14±0.04b | 20.45±0.06b | 23.54±0.01b | 6.27±0.03b | 12.43±0.03b | 9.26±0.03b |
| | 83 | 1.18± 0.03c | 1.06±0.06c | 16.32±0.06c | 19.73±0.04c | 5.06±0.03c | 16.55±0.04c | 5.15±0.05c |
| SD2 | 0 | 2.04± 0.01a | 3.95±0.02a | 23.48±0.04a | 25.99±0.04a | 7.03±0.03a | 9.44±0.02a | 9.43±0.01a |
| | 50 | 1.98± 0.02b | 3.51±0.04b | 21.15±0.05b | 20.01±0.04b | 5.22±0.03b | 15.54±0.03b | 8.36±0.04b |
| | 83 | 1.06± 0.03c | 2.34±0.04c | 14.01±0.05c | 18.25±0.03c | 3.62±0.05c | 17.10±0.03c | 5.06±0.03c |
| CS1 | 0 | 1.92± 0.02a | 4.02±0.03a | 26.90±0.02a | 23.45±0.03a | 8.05±0.03a | 12.45±0.01a | 7.11±0.03a |
| | 50 | 1.91± 0.02b | 3.51±0.03b | 23.15±0.04b | 22.73±0.02b | 7.00±0.02b | 14.04±0.02b | 6.68±0.05b |
| | 83 | 0.89 ±0.03c | 1.08±0.06c | 18.04±0.05c | 20.92±0.03c | 6.21±0.04c | 15.57±0.04c | 6.05±0.04c |
| CS2 | 0 | 2.05± 0.01a | 4.13±0.03a | 28.88±0.03a | 23.91±0.01a | 9.71±0.01a | 13.71±0.02a | 8.12±0.01a |
| | 50 | 2.04± 0.02b | 3.56±0.05b | 25.07±0.06b | 21.35±0.02b | 8.05±0.03b | 15.06±0.04b | 8.01±0.03b |
| | 83 | 1.27 ±0.03c | 1.72±0.04c | 20.50±0.06c | 19.75±0.05c | 7.24±0.02c | 16.54±0.03c | 7.32±0.05c |
| SS1 | 0 | 2.02± 0.02a | 4.68±0.03a | 32.78±0.03a | 28.53±0.02a | 8.43±0.04a | 14.10±0.03a | 12.16±0.02a |
| | 50 | 2.01± 0.03b | 3.36±0.03b | 26.40±0.04b | 27.92±0.03b | 6.04±0.02b | 19.60±0.05b | 11.20±0.02b |
| | 83 | 2.00± 0.03c | 2.05±0.04c | 18.66±0.05c | 24.68±0.04c | 5.00±0.04c | 22.10±0.02c | 9.42±0.03c |
| SS2 | 0 | 2.22± 0.01a | 6.57±0.02a | 36.88±0.04a | 29.01±0.02a | 7.45±0.02a | 11.36±0.02a | 11.74±0.03a |
| | 50 | 2.21± 0.02b | 4.52±0.03b | 28.53±0.05b | 28..03±0.02b | 5.26±0.01b | 17.09±0.03b | 10.18±0.05b |
| | 83 | 2.05± 0.03c | 2.30±0.05c | 14.82±0.05c | 26.18±0.03c | 3.81±0.03c | 22.50±0.03c | 9.42±0.04c |
| CV (%) | | 1.25 | 1.74 | 0.99 | 0.11 | 0.93 | 3.61 | 0.50 |

*Means in each column followed by the same case letter are not significantly different by Duncan's multiple range test (P<0.05, d f=5)

## Anatomical variation in the leaves

The frequency of the stomata on the leaf surfaces varied among the accessions and within the treatment protocols (Table 4). It could be observed that the accessions from the Semi-deciduous zone (SS1, SS2) showed reductions in the frequency of their stomata on both surfaces of their leaves under all the treatment protocols as compared to those from the Savanna zones, which showed a reduction between the normal and the medium stress protocols only. The sizes of the stomata were affected significantly by the imposed water stress as all the accession had a reduction in the stomata along the stress regimes. The differences observed in the number of stomata were small because the cells might have been formed already during the juvenile stage of the plants. Thus the effect of the stress imposition had a significant effect on the sizes of the stomata but not the frequency. The measured parameters exhibited a lower spread (CV(%)=0.02-1.97) among the accessions.

Both Shao *et al* (2008) and Ezin *et al* (2021) observed similar changes in stomata features in an earlier study on cowpeas. Stoma normally closes in environments that tend to draw water from plants (Hall 2004). This action could be speculated to be an attempt by the plants to prevent excessive transpiration. Costa *et al* (2008) attributed the phenomenon to an increase in cell turgor in the guard cells that in turn pulls in the thin outer walls of the stoma to close the pore. Stomata closed when the water deficit became apparent though the effects of other environmental conditions like humidity, temperature, and wind could not be ignored. Stomata closure may also occur when the water content of subsidiary cells decreases (Dragicevic 2011). This creates a water potential gradient between the subsidiary cells and the guard cells and thereby causing water the move out of the guard cells.

**Table 4. Means and S.D data of the leaf measurements of the accessions studied under the different water treatments**

| ACCESSIONS CODES | MOISTURE STRESS (%) | WIDTH OF STOMATA PORES/ x10µm | DIAMETER OF GUARD CELLS/ X10µm | FREQUENCY OF STOMATA | | WIDTH OF CUTICLES / X10 µm | |
|---|---|---|---|---|---|---|---|
| | | | | UPPER SURFACE | LOWER SURFACE | UPPER SURFACE | LOWER SURFACE |
| | 0 | 0.44±0.02a | 1.94±0.02a | 20a | 24a | 0.20±0.01a | 0.18±0.01a |
| SD1 | 50 | 0.21±0.02b | 1.82±0.03b | 19b | 21b | 0.21±0.01b | 0.19±0.02b |
| | 83 | 0.17±0.02c | 1.73±0.03c | 18c | 20c | 0.22±0.02c | 0.20±0.02c |
| | 0 | 0.42±0.01a | 2.09±0.01a | 19a | 21a | 0.20±0.01a | 0.18±0.02a |
| SD2 | 50 | 0.30±0.02b | 2.01±0.02b | 17b | 19b | 0.21±0.02b | 0.19±0.02b |
| | 83 | 0.21±0.02c | 1.93±0.03c | 16c | 18c | 0.22±0.03c | 0.19±0.03b |
| | 0 | 0.36±0.02a | 2.13±0.01a | 16a | 22a | 0.25±0.02a | 0.24±002a |
| CS1 | 50 | 0.22±0.03b | 2.05±0.02b | 15b | 21b | 0.27±0.01b | 0.25±0.02b |
| | 83 | 0.15±0.03c | 2.01±0.02c | 15b | 21b | 0.27±0.02b | 0.25±0.03b |
| | 0 | 0.38±0.01a | 2.12±0.02a | 17a | 21a | 0.26±0.01a | 0.26±0.01a |
| CS2 | 50 | 0.27±0.02b | 2.06±0.02b | 16b | 19b | 0.28±0.02b | 0.27±0.02b |
| | 83 | 0.20±0.02c | 1.97±0.03c | 16b | 19b | 0.28±0.02b | 0.27±0.03b |
| | 0 | 0.31±0.02a | 2.23±0.02a | 14a | 20a | 0.33±0.01a | 0.32±0.01a |
| SS1 | 50 | 0.23±0.02b | 2.17±0.02b | 13b | 18b | 0.35±0.02b | 0.33±0.03b |
| | 83 | 0.12±0.02c | 2.08±0.04c | 13b | 18b | 0.35±0.03b | 0.33±0.02b |
| SS2 | 0 | 0.30±0.02a | 2.01±0.02a | 13a | 26a | 0.33±0.02a | 0.33±0.02a |
| | 50 | 0.24±0.02b | 1.97±0.03b | 12b | 24b | 0.34±0.03b | 0.34±0.02b |
| | 83 | 0.14±0.03c | 1.91±0.03c | 12b | 24b | 0.33±0.01a | 0.34±0.03b |
| CV (%) | | 1.97 | 1.16 | 0.02 | 0.27 | 0.10 | 0.04 |

*Means in each column followed by the same case letter are not significantly different by Duncan's multiple range test ($P<0.05$, df=5)

The plants of the different accessions showed an increase in the thickness of their cuticle between the normal irrigation and the medium stress protocols (Table 4). The exception to this was found in the cuticles on the leaf surface of the accessions from the Semi-deciduous zone where the cuticle layer increased under increasing stress. The observed variations registered a lower spread of CV(%) of 0.1 and 0.04 on the upper and lower surfaces of the leaves respectively among the plants of the accessions with P> 0.05, n= 6. The increases in the width of the cuticles of the leaves between the normal and the medium stress protocols were expected since the epidermal cells were not over stressed at that degree of application. The cells may have been able to secrete more cutaneous substances to be added to what was already on the leaf surfaces. Under the severe stress regime, the epidermal cells may be speculated to have been weakened by the stress regimes and failed to produce additional cutin. Thus, apart from an accession from the Sudan Savanna zone (SS2), all the other plants recorded an increase or maintained their cuticle sizes between the medium and severe stress protocols (Table 4). The trend of variation in the width of cuticles could also, be attributed to the genetic characteristics of the accessions (Asharaf *et al* 2017). It was seen that the stress imposition did not register wider differences in the mean values of the cuticle among the accessions from the drier ecological zones as compared to those from the moist zone. Accessions from drier ecological zones possess thicker cuticles that help them to prevent excessive evapo-transpiration effects in their environment (Hall 2004). Arkoh (2010) observed a similar trend in the thickness of cuticles of the leaves of cowpeas grown under water deficit conditions and attributed the phenomenon to an increase in the modification of organs by plants to reduce water loss from their inner tissues.

## CONCLUSION

All the plants of the different accessions grew well under normal irrigation whilst reductions in the sizes of both the morphological and the anatomical features of the plants were recorded under the water stress protocols. The exception was found in the frequency of the xylem vessels in the stele of the plants and the cuticle cover of the leaves where there were significant increases in number and width respectively under the stress regimes. In all, vegetative growth of the accessions of *Vigna spp.* in Ghana was accelerated by the availability and increase in moisture content in soils, and the accessions could survive low water holding capacities and grow well especially, the accession from the Semi-Deciduous zone of Ghana.

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