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Water Stress: Morphological and Anatomical Changes in Soybean (*Glycine max* L.) Plants

Phetole Mangena

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Abstract

Water stress is one of the most important physiological stress factors that adversely affect soybeans in many critical aspects of their growth and metabolism. Soybean's growth, development and productivity are severely diminished, when soil or cell water potential becomes inadequate to sustain metabolic functioning. However, little has been done to gather comprehensive information regarding the specific changes that occur in water-stressed plants at the anatomical and morphological level. In this study, deviations in root growth, shoot growth, stomatal conductance, yield components and anatomical features are reported. Treatments with two levels of water stress imposed by reducing irrigation (once in 7 days or once in 15 days) revealed that, all cultivars (Dundee, LS 677, LS 678, TGx 1740-2F, TGx 1835-10E and Peking) were highly susceptible to prolonged water stress, exhibiting severe dehydration and death. A 15.0 and 30.0% survival frequency was obtained in plants irrigated once in 7 days; LS 677 and Peking, respectively. Unlike many other stresses, water deficit did not only affect the density of stomata, but, photosynthesis was affected by the lower levels of tissue CO₂. These results suggest that, balanced biochemical, physiological, anatomical and morphological regulations are necessary for increased growth and yields in soybean.

Keywords: anatomy, growth, morphology, soybean, water stress

1. Introduction

Water stress is one of the most important constraints in the growth and development of plants. Water deficit stress, in particular, is a major problem in agriculture and most crop plants show high sensitivity to this kind of stress than any kind of abiotic constraint conditions. Crop plant growth and yields are severely impacted by inadequate supply of water, which result in decreased carbon assimilates contents. In addition, plants exposed to prolonged shortage

in ground, surface or atmospheric water, known as drought are highly susceptible to pests and diseases. Mattson and Haack [1] provided evidence on the occurrence of fungi and insect induced stalk rots, wilts and foliar diseases in plants caused by drought stress. The prevalence in disease outbreak occurred in water-stressed plants compared to the normal water stress-free plants. Estimations of yield losses in soybeans compiled by Wrather and Koenning in the United States from 1996 until 2007 indicated that, the role of pathogens such as soybean cyst nematode, phytophthora root and stem rot, as well as charcoal rot that affected seedling development was exacerbated by the physical environmental stress conditions [2]. Drought is, and continues to be an insidious hazard to plants, animals and human lives. Drought conditions in many regions worldwide are worsening due to various factors, some of which are caused by climate change. The increase in atmospheric CO₂ level, currently estimated at about 380–400 ppm, and alterations in hydrological cycles make drought a recurring natural hazard world-wide [3, 4]. In this regard, plants undergo permanent or temporary damage to their morphological architecture, and their anatomical and physiological processes when exposed to dry and hot conditions. According to Shao et al. [5], water stress effects can be extended in plants to alter gene expression, change cellular metabolism, cause reduction in mitotic cell division activities in mesophyll tissues and other organs, as well as to cause the decrease in stomatal conductance [6]. Scientific research showed that; drought stress causes imbalances in the natural status of the environment and drastically disrupts crop cultivation thus, threatening food security [7, 8]. Many regions have experienced the detrimental and severe effects of drought, particularly, populations in the developing countries. In the Southern African Development Community (SADC) region; poor rainfall conditions were recorded for the 2016/2017 agricultural season as a result of El Niño induced drought [9]. FAO's global information and early warning system in 2015 reported significant drought dating back to 1984 [10]. The area data covered regions such as the United States, Semi-Arido of Brazil, Eastern Europe and African countries where, severe drought causing food crisis across Ethiopia, Kenya and Somalia resulted into the deaths of over 1 million people. Therefore, the continuing drop into below-normal annual rainfalls and increasing temperatures create the relevance to study and understand the morphological/anatomical changes that plants undergo to cope with environmental stresses. In cultivated crops such as soybean (*Glycine max* L.), this would minimise limitations that adversely affect plant growth, and the improvement of this crop for yield purposes [11], as well as counteracting against factors that negatively influence the nutritional content and essential secondary metabolites synthesised in this plant.

2. Analyses of soybean responses to water deficit stress

Plants experience water deficit stress when the amount of water in the cells and surrounding becomes limiting to growth and development. To investigate these effects, a study was conducted to primarily assess the influence of water stress on the growth of soybean; morphologically and anatomically, under greenhouse conditions. According to Lisar et al. [8] water deficit is caused by prolonged water shortage. In order to examine this stress, reduction in the frequency of irrigation was performed by limiting watering to once a week (WT 1) and once

in 15 days (WT 2). Plants used for the control were watered daily, depending on soil moisture content in the plastic pots. For the growth of soybean plants, plastic containers filled with a mixture of 4:1 (v/v) fertile sandy-loam soil with vermiculite was used. Seeds of soybean cultivar Dundee, LS 678, LS 677, TGx 1740-2F, TGx 1835-10E and Peking were inoculated into the pots for germination and seedling emergence. At least one soybean plant was grown per pot with 20 replicates per cultivar, and allowed to grow up to V3 stage before imposing water stress. The morphological and physiological data were then recorded, which included plant height, number of leaves plant⁻¹, number of braches plant⁻¹, yield and yield components, average leaf area, root length and the micro-morphological characteristics of the stomata and trichomes were evaluated. Assessment of these characteristics was guided by the methods according to Cornelissen et al. [12] with modifications. To study stomatal and trichomes characteristics the microscopic slides were prepared by a protocol modified from Yeung's [13] guide to study plant structures. Leaves of soybean plants from both the control and water-stressed plants (WT 1 and WT 2) were collected a week before the experiment was terminated. The experiment was terminated when the plants reached reproductive stage 4 (R4) of fruiting, involving maturity and seed filling. The free hand sectioning method by Yeung was used to study structural organisation of the root and stems, with section staining done using Toluidine blue O stain. Quantifying the chlorophyll content and leaf area is an important measurement for comparing plant growth, treated with different growth conditions. For leaf area assessment, leaf samples were randomly detached from the different cultivars, and their leaf area estimated as described by Richter et al. [14]. Leaves were randomly sampled for estimation of chlorophyll content using a CCM 200 plus Chlorophyll Meter, Opti-Sciences.

3. Description of soybean morphology and anatomy

Plants are responsible for a number of essential ecological services. Plants are the main primary source of foods for humans and animals, supply oxygen, timber, medicine and also have ornamental value. The multiple and complex processes involving genetic, morphological, anatomical, physiological and biochemical mechanisms are responsible for the goods and services that plants provide. These functions are made possible by the architecture of the plant's internal and external structures. Soybeans like other legumes and non-leguminous plants display different types of internal and external growth forms that functions together to provide these services. The external form include indeterminate, determinate and semi-determinate morphological growth habits, which typically take place in both the early and late maturity groups of varieties grown for commercial and subsistence farming [15]. Soybean plants with determinate growth terminate their vegetative growth stage during the onset of the reproductive stage. In contrast, indeterminate varieties continue growing even during flower setting and anthesis. Anthesis is the period in which flowers developed during the reproductive stage of the plant's life cycle begin to open. According to the NDSU [15] the semi-determinate growth habit lies between the polarity and growth of the other two growth habits (determinate and indeterminate form). The vegetative parts of soybean include the stem, leaves and the soil submerged roots. A few types of leaves can be found in soybean.

The plant has trifoliate leaves, which are photosynthetic foliage with three leaflets. They have protective scale leaves which covers and protect young immature flowers before anthesis.

These scales are small bracts which appear subtending the yellow or purple flowers of soybeans [16]. The special leaf types constituting the floral parts or inflorescence (raceme) can also be found. The vegetative stage is furthermore characterised by erect elongated stems, axillary buds, some viewed immediately above the cotyledons at the axil, unifoliate buds and the terminal buds (**Figure 1a–c**). Both young and old stems of soybeans are heavily covered by the epidermal hairs (trichomes) (**Figure 1d**). Even though soybean plants produce primary roots, originating from the seedling's embryo; the roots have many branching secondary roots that slightly resemble fibrous root system in monocots. Most of the lateral roots are concentrated at the upper part of the root zone. As in most of the dicotyledonous plants, soybean's body is made up of the three main tissue systems: dermal, ground and vascular (**Figure 1e, f**). The epidermis as the dermal tissue is the

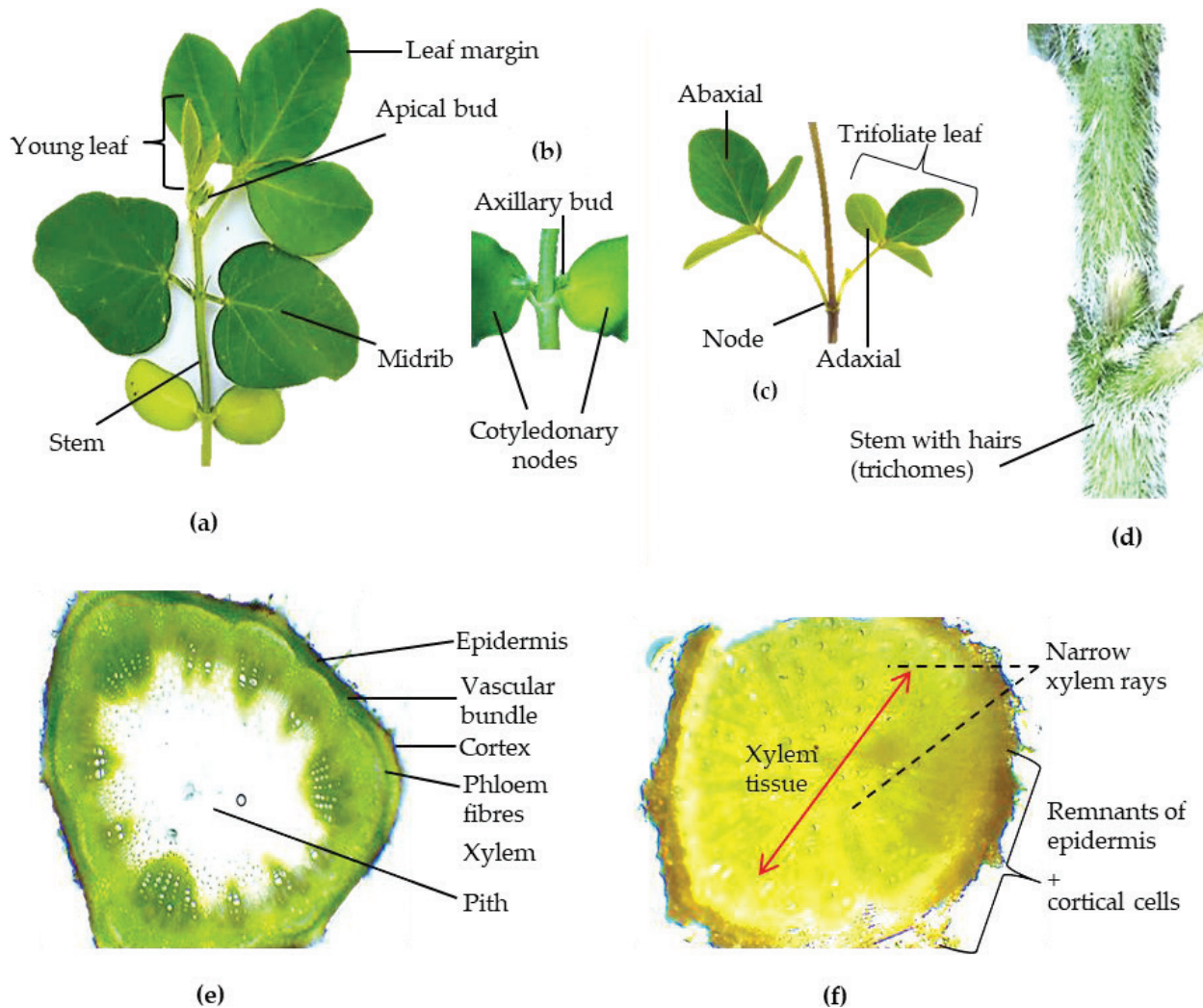
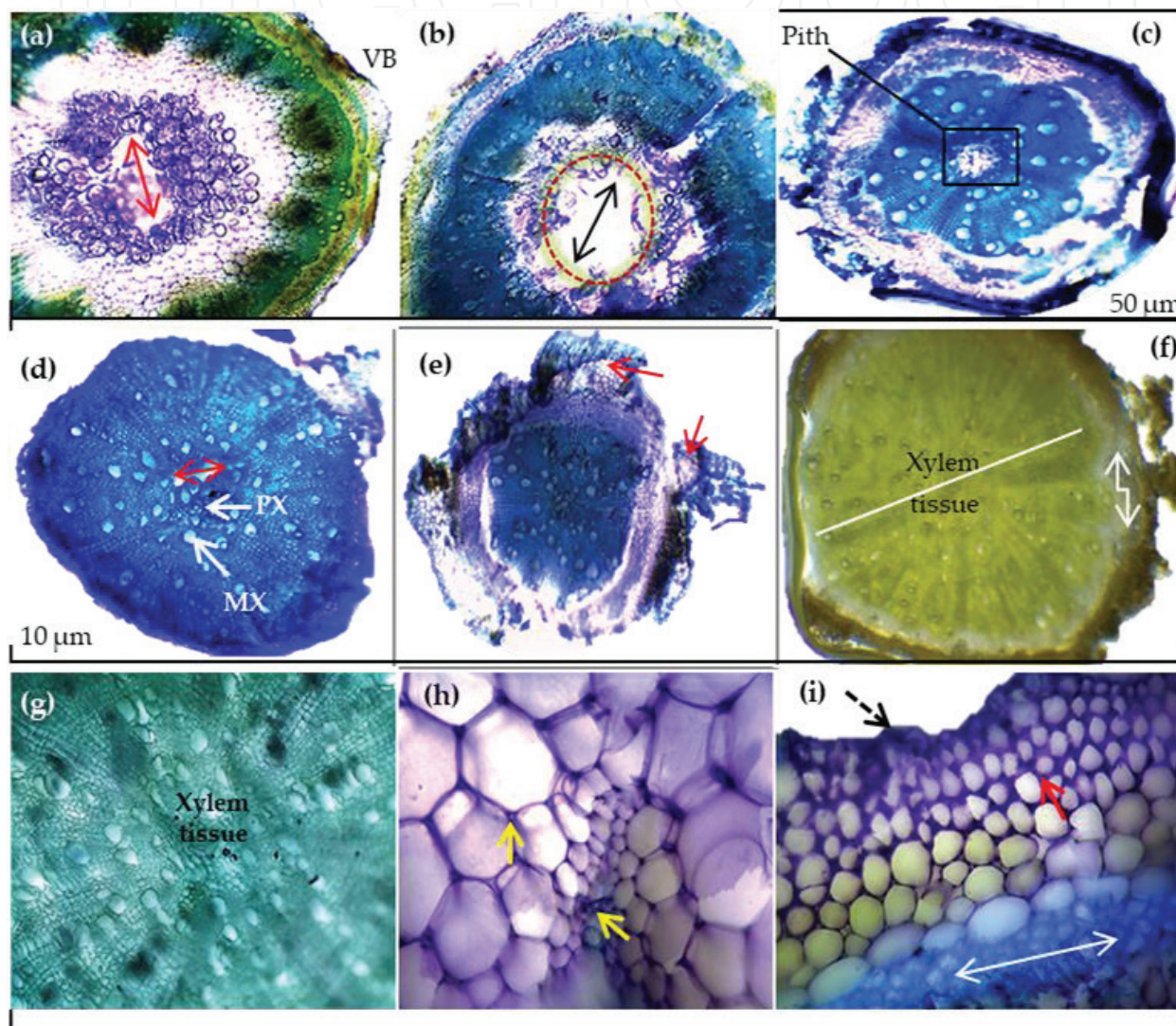


Figure 1. Overview of soybean plant morphology and anatomy. (a) Vegetative first trifoliate (V1) stage. (b) Example of cotyledons and axillary buds at the axil. (c) Trifoliate leaves showing adaxial-abaxial leaf surfaces. (d) Example of soybean stem with epidermal hairs. (e) A micrograph of soybean stem cross-section. (f) A micrograph of soybean root cross-section.

outermost single layer of cells derived from the protoderm, and in soybean it covers the plant for its entire life cycle. The three main types of epidermal cells found in soybean include trichomes and microscopic guard cells as well as the subsidiary cells of the stomata (Figure 2b–d). This layer of elongated and compactly arranged cells functions to protect soybean against water loss and harsh external environmental factors, including pathogens. Trichomes are unicellular or multicellular hairs occurring on shoot system of plants. On the roots, hairs are called root hairs. In leaves, this layer of cells is followed by the palisade parenchyma and spongy mesophylls.



50 μm

Figure 2. Examples of microscopic cross-section in roots and stems of soybean plants. (a) Formation of pith canal as a result of water stress in WT 1 plants. (b) Broadening of canal and changes on stem cortex tissue in WT 2. (c) Control plants showing unaffected pith and cortex. (d) Cross-section of WT 2 root showing rupturing of the stele, protoxylem (PX) and metaxylem (MX). (e) Cross-section of WT 1 root showing xylem marks of lateral roots (left right arrow). (f) Root section taken from the control showing expanded thickened xylem tissue and reduced cortex. (g) Close view of xylem tissue from the control plant. (h) Close view of parenchymatous pith as indicated on (c), (arrows indicate intercellular spaces of the parenchyma). (i) Soybean cortical tissue of the stem showing phloem fibres (left right arrow), collenchyma (solid arrow) and a single layer of epidermis (dashed arrow).

The palisade and spongy cells are specialised tissues used by all eudicot plants with C_3 pathway for photosynthesis and gaseous exchange in leaves, respectively [17]. Soybean is one of the C_3 plants which undergo photosynthetic carbon reduction and do not have a CO_2 concentrating mechanism. It differs with grain crops such as maize, rice, sorghum and wheat C_4 plants that concentrate CO_2 by not salvaging carbon lost during photorespiratory carbon oxidation (PCO) cycle [17]. But, the palisade and spongy tissues of soybean form the mesophyll, a ground tissue system of a leaf, which plays a critical role in carboxylation, reduction and regeneration processes during photosynthesis. In roots and stems, the fundamental (ground) tissue consists of non-protective and non-conductive simple cells of parenchyma, collenchyma and sclerenchyma (**Figure 2e** and **f**). Evert and Eichhorn [18] referred to this tissue system as the one most dominated by parenchyma cells, which are by far considered the most common ground tissue of the pith and cortex in roots and stems of soybean and other eudicots, as well as in the monocots. The vascular system is made up of conducting strands of phloem and xylem. These are principal water and food conducting tissue in all vascular seedless and seed plants.

3.1. Morphological changes due to water deficit stress

The morphological evidence gathered in this study has shown that soybean growth is highly sensitive to water deficit stress. All plants exposed to water deficit presented significant changes in their shoot and root morphology. Complete reduction in the number of new branches per plant, initiation of leaves and expansion of the lamina (measured by estimated leaf area) and the number of trifoliolate leaves per plant was observed. Decreases in the assessed morphological characteristics were more predominant in plants subjected to stress for longer periods (WT 2) than those watered once a week (WT 1). Soybean cultivar Dundee, TGx 1740-2F, TGx 1835-10E and Peking produced significantly similar mean number of trifoliolate leaves (about 4.0–5.0) in WT 2, when compared to about 5.0–6.0 trifoliolate leaves obtained in WT 1 (**Table 1**). Leaf rolling and flipping were observed in some of the older leaves as a result of induced water stress. The negative effects of water stress on new leaf and branch formation was also reported by Mambulwana [16]. Jaleel et al. [19] similarly added that, water stress decreases the elongation and expansion of stems and leaves. In contrast to observations made in all water stressed plants, the control exhibited normal shoot growth and the highest number of trifoliolate leaves (**Table 1**).

According to Nosalewicz and Lipiec [20] suppression on the growth and distribution of the roots by water stress could also lead to the reduction in shoot growth. As the vegetative shoot growths appeared diminished by induced stress, roots in water-stressed plants became more elongated and branched than in the control. Root phenotype in the control appeared shallow and less branched than in WT 1 and WT 2 plants. However, plants which had irrigation reduced to once in 15 days (WT 2) had deep root phenotype compared to plants irrigated once a week (WT 1). Insufficient water supply for WT 2 plants with deep root development, and moderately stressed plants (WT 1), both demonstrated clear morphological changes. All cultivars in WT 2 also exhibited severe nutrient deficiency symptoms (the entire leaf with chlorosis and marginal necrosis) and stem wilting. These symptoms were accompanied by adverse growth effects and survival frequency of 0% when the experiment was terminated (**Table 2**). Water deficit stress ultimately led to the severe damage to shoots of WT 2 plants, with no possible indication of recovery. In WT 1 plants, moderate to severe deficiency symptoms

Soybean genotypes	Treatment plants 1			Treatment plants 2			Control plants		
	Mean no. of fully developed trifoliolate leaves	Average leaf area (cm ²)	Stomatal density (no. of stomata/ cm ²)	Mean no. of fully developed trifoliolate leaves	Average leaf area (cm ²)	Stomatal density (no. of stomata/cm ²)	Mean no. of fully developed trifoliolate leaves	Average leaf area (cm ²)	Stomatal density (no. of stomata/ cm ²)
Dundee	5.0 ^a	55.1 ^a	213 ^a	5.0 ^a	55.1 ^a	112 ^a	13.0 ^a	50.0 ^a	247 ^a
LS 677	7.0 ^b	38.6 ^b	191 ^b	6.5 ^b	32.1 ^b	106 ^b	14.5 ^b	41.1 ^b	213 ^b
LS 678	6.0 ^c	40.1 ^c	203 ^c	6.0 ^c	32.1 ^b	167 ^c	13.5 ^c	57.7 ^c	212 ^c
Peking	8.0 ^d	43.9 ^d	181 ^d	4.5 ^d	36.5 ^c	143 ^d	15.5 ^d	33.6 ^d	256 ^d
TGx 1740-2F	6.0 ^c	37.5 ^e	154 ^e	5.0 ^a	40.1 ^d	163 ^e	12.5 ^e	16.1 ^e	163 ^e
TGx 1835-10E	6.0 ^c	60.1 ^f	167 ^f	4.0 ^e	30.7 ^e	155 ^f	11.0 ^f	39.8 ^f	171 ^f

The leaf area of central individual leaflets in soybean cultivars were estimated using the general Eq. $LA = k \times (L \cdot W)$ where LA, leaf area; k, is the 'adjustment factor' estimated by linear regression forcing the regression intercepting line to be zero using Table Curve software (Richter et al. [14]), L, length of the leaflet and W, leaflet width.

Values within columns followed by different alphabets are statistically significant at $p \leq 0.05$ confidence level. For Water Treatment 1 (WT 1), irrigation frequency was reduced to once a week (After 7 days), Water Treatment 2 (WT 2); reduced to once in 15 days and the Control, watering depended upon moisture availability in the soil.

Table 1. Developmental patterns in the leaves of water stressed and unstressed soybean plants measured immediately after the termination of the water deficit stress experiment.

Soybean genotypes	Mean plant height (cm)		Mean no. of branches		Flowering plants (%)		Mean no. of pods produced		Survival frequency (%)	
	TP 1	TP 2	TP 1	TP 2	TP 1	TP 2	TP 1	TP 2	TP 1	TP 2
Dundee	25.2 ^a	24.1 ^a	3.0 ^a	3.0 ^a	—	—	—	—	—	—
LS 678	40.0 ^b	26.4 ^b	3.0 ^a	3.0 ^a	—	—	—	—	—	—
LS 677	33.3 ^c	26.5 ^b	4.0 ^b	3.0 ^a	1.00 ^a	—	7.0 ^a	—	15.0 ^a	—
Peking	24.2 ^d	23.5 ^a	4.0 ^b	2.0 ^b	15.0 ^b	—	3.0 ^b	—	30.0 ^b	—
TGx 1740-2F	27.3 ^e	21.0 ^c	2.0 ^c	3.0 ^a	—	—	—	—	—	—
TGx 1835-10E	26.1 ^f	20.7 ^d	3.0 ^a	3.0 ^a	—	—	—	—	—	—

Percentage survival frequency was calculated from the number of plants/ genotype that survive until the termination of the water stress deficit experiment.

Statistical significance among the values is designated by different superscript letters. Values within columns showing different letters are statistically varied (at 0.05) by ANOVA.

Table 2. Vegetative growth and flowering response of soybean plants subjected to water deficit stress conditions.

were observed. Soybean cultivar LS 677 and Peking showed some resistance with 15 and 30% survival rate (**Table 2**). A few plants in these two genotypes exhibited moderate stress effects among all the cultivars assessed. There were no differences in the lengths of root system and shoots observed in water stress resistant cultivars (LS 677 and Peking) in comparison with those severely affected (Dundee, LS 678, TGx 1740-2F and TGx 1835-10E) in both WT 1 and WT 2 plants.

Klamkowski and Treder [21] reported almost similar results in water stressed strawberry plants. In addition, there were no major differences, especially in root lengths that were observed between water stressed plants and the control. The report cited inhibition of growth by water stressed plants, involving decrease in root expansion as suggested by Boyer [22]. This claim probably led to the observed root phenotype in water stressed strawberry plants. This is in contrast with finding in this study and most of the other suggestions made on root phenotypes during water stress. In general, root formation has been found to increase in length during water stress, with roots growing deep into the soil in search for moisture [17, 19, 23, 24]. This further development in the root system is an attempt by plants to increase the uptake of water in order to sustain growth as observed in this study.

3.2. Anatomical changes in response to water stress

The WT 1 and WT 2 plants demonstrated a different internal anatomy compared to the control plants. Stem cortex of water stressed plant were generally smaller compared to the cortex in stems of plants in the control (**Figure 2**). However, vascular tissue thickening and expansion was observed in both the roots and stems of water stressed and control plants. The development of the secondary tissues in water stressed plants, especially the deposition of secondary xylem cells (as viewed in **Figure 2a, b**), was interrupted by the gradual rapturing of the pith which resulted in the formation of pith canals. Pith canals are hollow centres, called central

canals, which are usually formed in woody shrubs and trees. They are formed when the earliest vascular tissues, protoxylem, is destroyed by the formation of new metaxylem as the root or stem grows in diameter. In gymnosperms, these canals are instead used by the pine trees to store resin and they are more associated with the cortical tissue of the stems than the pith [25]. In stems of plants such as seedless vascular plants (horsetails), these canals are naturally formed to reduce the weight of the stem thus, increasing stem strength and resistance to buckling [18]. However, the formation of canals (breaking down of the soybean pith tissue) observed in roots and stems may have resulted from water stress. Furthermore, this may have possibly impacted negatively on the growth of plants, particularly when induced as a result of severe water stress, like in WT 2.

Even though the pith is poor in nutrients [26], the parenchyma cells can still function in storage of nutrients and water for the plant. Pallardy [27] suggested that, rapturing could also destroy the interconnectivity between the storage parenchyma of the pith with the cortex, disrupting short distance transport that occurs through the rays via secondary xylem. The variations in canal diameters between WT 1 and WT 2 (**Figure 2a, b**, including canal in the root- **d**) may be in response to the different water stress regimes or the genotype variability of the soybeans used. Canal diameters in soybean WT 2 plants were larger than the diameter observed in WT 1 plants (**Figure 2a, b**). Soybean cultivar LS 677 and LS 678 showed little resistance to the rapturing of the pith, compared to cultivar Peking and Dundee. This was the case, even though cultivar LS 677 and Peking were the only varieties more resistant to water stress treatment (WT 1). This could be both a genetically-linked response and the reaction or effects of water stress conditions to the tissue development. In cultivar Peking, TGx 1740-2F and TGx 1835-10E, pith canals appeared to be continuously cut from the central pith further to the cortical cells. This induced complete disruption of water transportation through some part of the xylems, xylem rays and nutrient transport by the phloem tissues. The cutting of water supply may have resulted in the poor survival rates observed in most of the cultivars (**Table 2**). But, the absence of pith canals in stems of the control plants furthermore suggests a relationship between water deficit and the change in anatomy of the soybean plants. When the imposed environmental stress reduced the rate of tissue development, the length of xylem rays in roots was also reduced.

The reduction occurred when growth is affected by death of tissues and slowing down of metabolism as a result of the stress. Alteration in plant metabolism affect cell division, thus cell elongation and expansion is negatively affected as evidenced in **Figure 2d, e**). The xylem cell portion in the roots of water stressed plants was reduced compared to xylem tissue diameter in the control. Yamaguchi and Sharp [28] indicated that, water stress induce changes in root growth and cell length distribution which may be directly related to growth inhibition in roots, especially at root elongation zones. Another example is by Schuppler et al. [6] who also indicated the reduction on mitotic activity of mesophyll tissues in wheat (*Triticum aestivum*) seedlings subjected to mild water deficit. These reports indicate that, the lack of adequate water supply decreases the rate of cell division and tissue expansion in all plant organs, although root morphology may appear less affected in contrast to root anatomy. Munns and Sharp [29] made similar remarks following their investigation on the effect of abscisic acid (ABA) on shoot and root growths during salinity and drought stress.

4. Effect of water deficit on plant metabolism

The synthesis and breakdown of metabolites to yield energy is required for the many activities that plants depend upon. But, when plants are exposed to drought stress conditions, physiological and metabolic changes occur. Immediate acclimatisation by the alteration of plant morphology is therefore required for plants to be adapted to the changing environments. Whether plants succeed to acclimatise or not, the subsequent phenotypic modifications observed in water stressed plants would be a function of the metabolic changes. In soybean, like other leguminous plants, decrease in the leaf area, number of individual leaves and the total number of branches per plant is normally observed [16, 24]. However, on the metabolic section, water stressed plants experiences a dramatic decrease in photosynthetic rates as a consequence of the modification in photosynthetic structures and chloroplastidic pigments. Chloroplastidic pigments involve all plant pigments such as chlorophylls and carotenoid pigments embedded in the thylakoid membranes of parenchyma mesophylls [18]. These pigments are primary molecules responsible for making sure that light energy from the sun is captured and converted to chemical energy required for metabolism.

This is the main route in which energy used for synthesis of biological products enters our biosphere. Water stress adversely limits this process by inhibiting the functioning of structure serving as primary support for photosynthetic metabolism. According to Kwon and Woo [24] drought reduce photosynthesis by limiting stomatal operations. In line with this report, the soybean plants subjected to water stress (WT 1 and WT 2) kept their stomata closed to reduce transpiration, hence trying to preserve water. The stomatal micrograph in **Figure 3** illustrates closed stomatal apertures (c, d) prepared from leaves collected during the day. The closure of stomata in turn reduces the concentration of CO₂ required in the mesophyll for carboxylation process during the manufacturing of photosynthates. This phenomenon was also reported by Dekov et al. [31], Evert and Eichhorn [18], Lopez-Carbonell et al. [30] and Taiz et al. [17]. Additionally, there were significant variations in stomatal density exhibited by the different genotypes.

Water stressed soybean cultivar TGx 1740-2F and LS 677 exhibited low density of stomata with an average of 154 and 106 in WT 1 and WT 2 respectively, among all the cultivars used (**Table 1**). Furthermore, the two TGx cultivars (TGx 1740-2F and TGx 1835-10E) did not show extensive variations in the stomata among all water stressed plants, including the control. The mean leaf areas of the water stressed plants were also significantly lower compared to the control. The decrease in the leaf area of the plants posed negative effects on the rate of photosynthesis by reducing the leaf surface area in which light is captured. Anatomically, water stress also had an effect on leaf mesophyll thickness which also had an impact on photosynthesis. Cramer and Browman [32] attributed this to the changes in the rate of cellular expansion, which was observed in the maize mesophyll tissues when cell division and differentiation appeared affected by drought stress. However, plants growing in soil grounds of very lower water potential possess poor cell formation and expansion. Schuppler et al. [6] also reported this when assessing the effects of water stress on rate of cell division or mitotic activity on wheat leaf tissues. The report indicated that generally, leaf tissue expansion rate is reduced to more than 50% when plants are subjected to drought stress. In terms of physiological response to water stress, the reduction in chlorophyll content index (CCI) in water stressed plants was

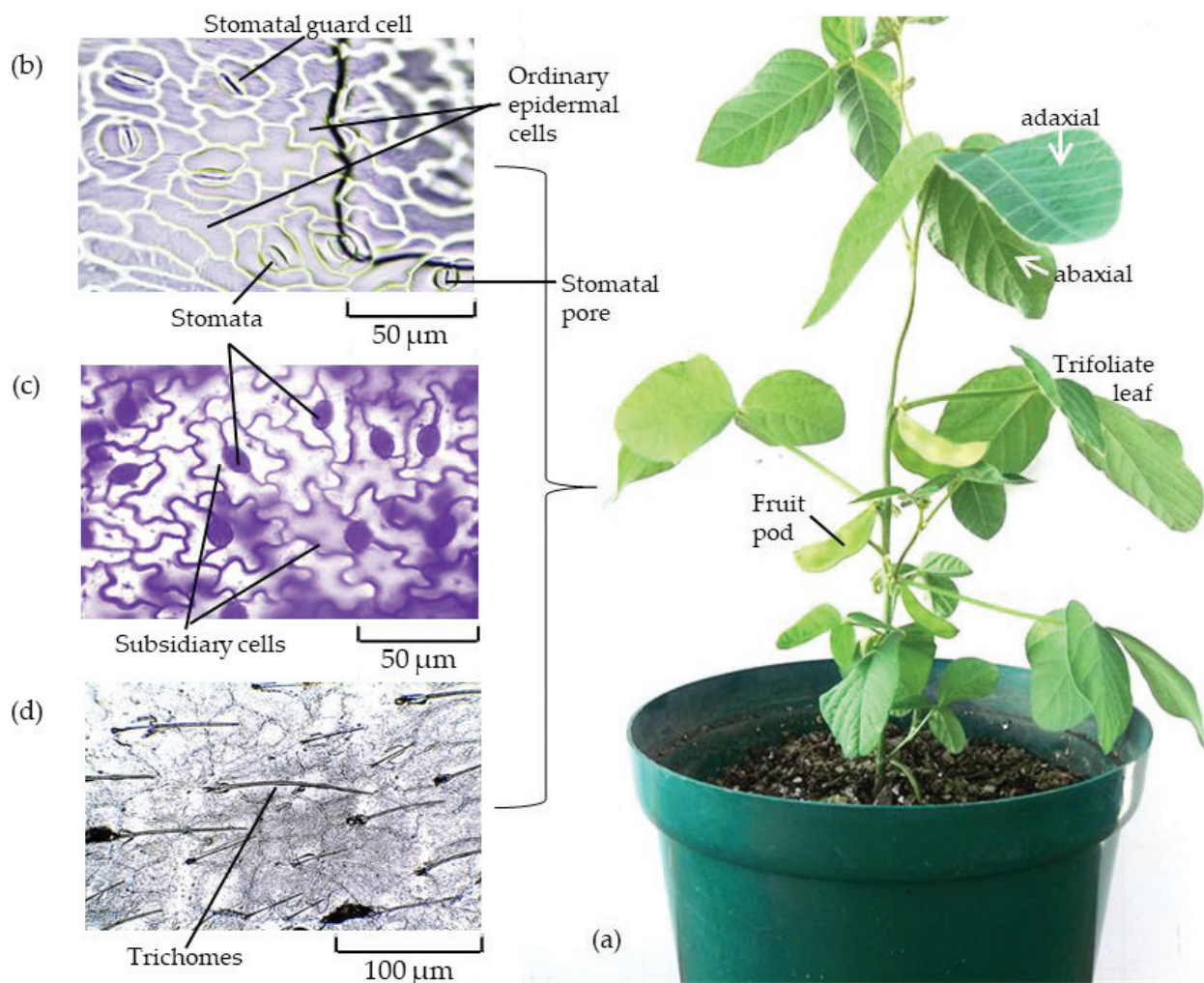


Figure 3. Dermal tissue of the leaf of a typical soybean plant. (a) Soybean plant at R4 stage. (b) Field of epidermal cells of a soybean plant. (c) Light micrograph of slightly higher magnification of stomatal complexes on a soybean leaf. (d) Light micrograph in the epidermis showing epidermal hairs.

recorded, and the decreases in chlorophyll contents varied according to imposed water stress treatments (**Figure 4**). Plant irrigated once in 15 days (WT 2) showed remarkable decrease in CCI (**Figure 4d**) than WT 1 plants (**Figure 4b**).

Control plants did not exhibit significant reduction in CCI nor variation in all cultivars' CCI measurements even before when water treatments were imposed on water stressed plants (**Figure 4a, b**). But then, differences were not expected in the CCI estimates of control plants measured early during growth and later before termination of the experiment, since the plants were adequately watered. Therefore, as expected the chlorophyll degradation was not induced on control plants as a result of water stress. As the differences in the chlorophyll content and degradation were observed in water stressed plants, these findings were in line with Dhanda et al. [33] and Benjamin and Nielsen [34]'s reports on the effects of drought on plant metabolism. As indicated on Section 2, to examine and confirm the degradation of chlorophyll and its subsequent effects on photosynthetic activity, starch analysis was performed. Leaves detached from randomly selected soybean plants

were obtained and taken to the laboratory for starch analysis. The leaves were bleached in boiling 90% ethanol and incubated in dilute iodine (0.5 M) solution (2:1) for 3 minutes and then rinsed with distilled water. Rinsing is necessary to remove excess iodine solution

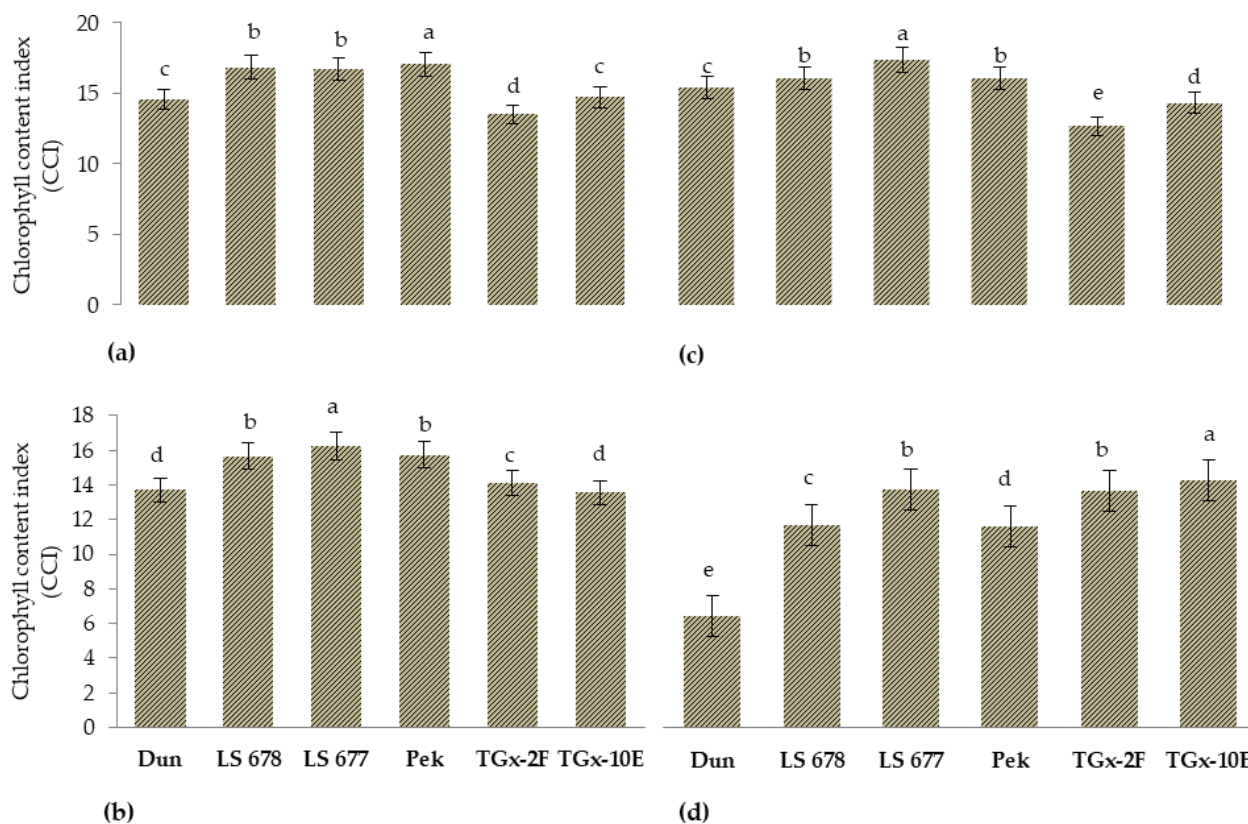


Figure 4. Effect of water deficit stress on photosynthetic pigment (chlorophyll) content of soybean plants expressed to CCI. (a) Chlorophyll content of control plants during early growth stages (V3). (b) Leaf chlorophyll content of the control during early reproductive stages. (c) Amount of chlorophyll content in WT 1 plants. (d) Leaf chlorophyll content in WT 2. Data represent CCI means and the different letters denote significant differences of the means at $p < 0.05$.

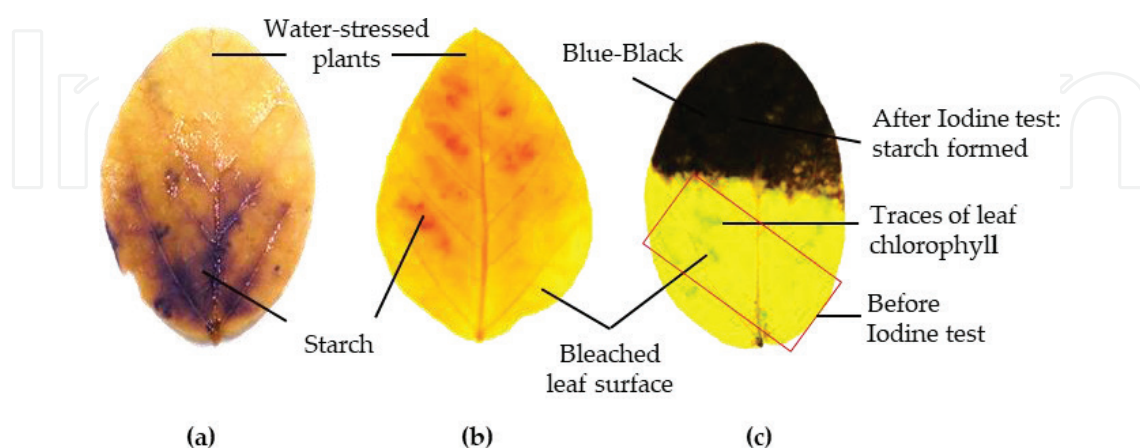


Figure 5. Iodine test on ethanol bleached leaves. After bleaching and staining with iodine: (a) Show traces of starch on leaflet taken from water stress plants (WT 1). (b) Absence of or minor starch traces on severely water stressed leaflet (WT 2). (c) Starch content (blue black colour) on leaflet taken from the control plants.

on the leaves while a colour change occurs. The iodine stained leaves (**Figure 5a–c**) were then visualised under a ZIESS Discovery V12 stereo microscope mounted with an ICc5 Axio-Camera. The presence of high starch content was observed in the control (**Figure 5c**); whereby starch contents in WT 1 (**Figure 5a**) and WT 2 (**Figure 5b**) were very drastically reduced because of poor photosynthetic activity. Intense blue-black colour on the leaves of control plants indicate the presence of starch, generated from the photosynthesised carbohydrates. Only minor traces of starch were observed from WT 1 and WT 2 leaves due to water stress.

5. Nodulation

The formation of cell protuberance containing nitrogen-fixing Gram-negative bacteria in the roots of legumes plays an important role in improving plant growth characteristics, crop productivity and maintaining soil fertility. This establishment of lumps on roots of plants (known as nodulation) guarantees the supply of fixed atmospheric N_2 for use in the synthesis of proteins, nucleic acids and other necessary nitrogen-containing compounds required for plant, animal and human growth and development. However, various reports have indicated that, water stress induces low frequencies of nodulation in many legumes, including soybean. Miao et al. [35] provided evidence that verifies sensitivity of soybean nodulating root cells and *Rhizobium* to water stress. In 2003, Ramos et al. [36] also indicated that, water stress affect nodulation in other legume species like *Phaseolus vulgaris* L. Failure for soybean roots to produce effective nodulations affect the metabolism of nitrogenous and carbonic compounds in the plant. The changes resulting into decreased nodulation could cause reduction in various aspects of plant growth (stem height, stem wood diameter and root dry weight) due to drought as reported by Shetta [37]. Additionally, Shetta indicated that the initiated nodules can become thickened and more resistant to infection by *Rhizobium* as a result of this stress. Poor nodulation can be induced by poor plant nutrition, seed filling, or abiotic stress factors. In WT 2 plants, where irrigation was withheld for 15 days, nodulation was severely affected (**Figure 6f**). It was found that nodules stopped fixing nitrogen and then started decomposing. Nodulation and nitrogen fixation in the WT 1 also decreased following imposed water deficit stress. The nodules turned green (**Figure 6e**) and this predominant green colour indicated inefficient fixation by *Rhizobium* strain in contrast to highly efficient red-pinkish nodules in the control (**Figure 6d**). This inefficiency may have been caused by the poor amounts of assimilates that are exchanged from soybeans to the bacteria due to reduced rates of photosynthesis in the leaves. Plants do not get fixed nitrogen from *Rhizobium* for free. For plants to receive fixed atmospheric nitrogen, in a form that is directly available for growth (nitrates– NO_3^- and ammonium– NH_4^+), plants must give bacteria sugars. This symbiotic relationship was reported by Dupont et al. [38], Serraj et al. [39] and Stajkovic et al. [40] as the major stimulant of increased plant biomass, stabilising atmospheric CO_2 by stabilising C–N ratio. The symbiosis establishment is playing a very critical role in ecological and agronomic supply of N_2 , estimated to account for a total of about 65% of the nitrogen fixed in legumes used for agriculture globally.

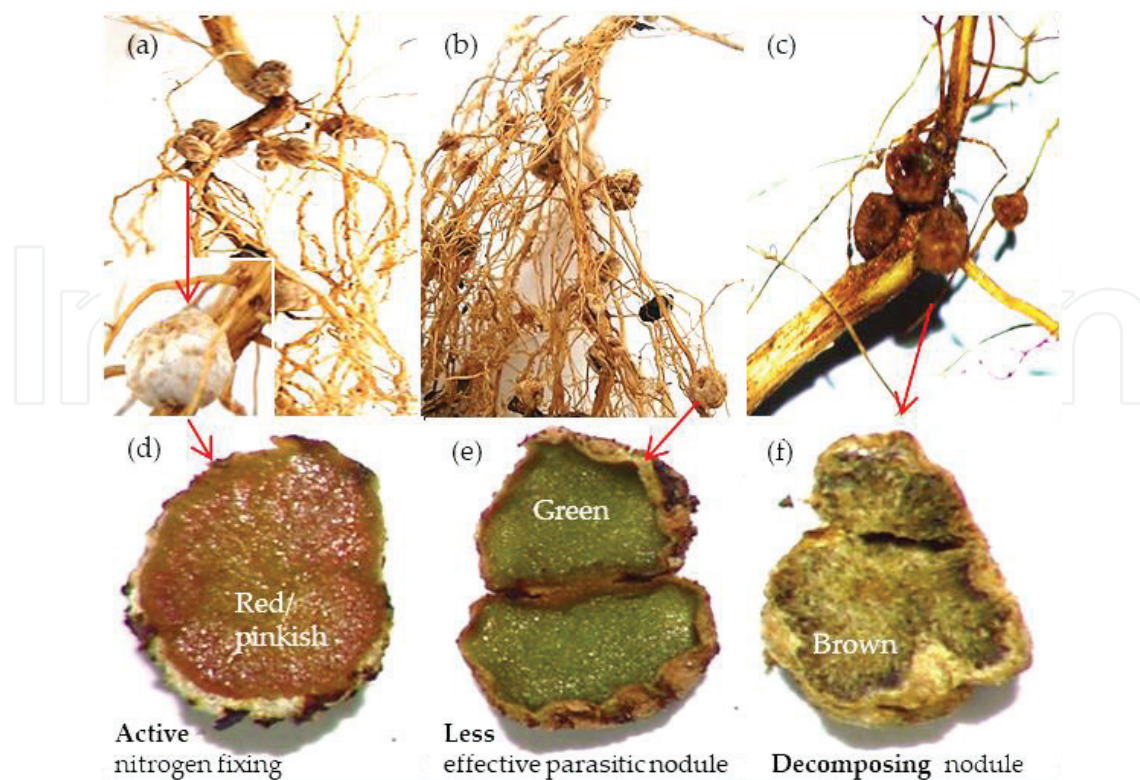


Figure 6. Soybean plants with nodulated roots. (a) Healthy nodules on soybean control plants. (b) Roots of WT 1 with numerous mature nodule structures. (c) WT 2 stressed plant root showing poor nodulation. (d) Nitrogen (N) fixing nodules with *Rhizobia* as observed in the control. (e) Less effective nodules from WT 1 roots. (f) Decomposing root nodule of WT 2 plants.

6. Impact of water deficit on flowering and fruiting

The soybean genotypes showed great differences in the percentage flowering, pod formation and other yield related components. Water stressed plants produced less than 2% yield, in the two soybean cultivars (LS 677 and Peking) that survived induced water stress. A few WT 1 plants subjected to water stress continued their growth until flowering and pod formation stages. However, flower and fruit pod abortions were simultaneously observed leading to 7.0 and 3.0 mean pod number observed in the few plants that had survived (**Table 3**). These numbers were not comparable with the yield component data recorded for these cultivars in the control. Soriano et al. [41] determined a positive relationship between yield quantity by estimating grain number and weight in early planted sunflower by timing induction of environment stress. In line with this report, positive yield characteristics that include; total percentage of flowering plants, mean number of pods and average pod length, pod weight and seed weight (per 100 seeds) were observed in all of the cultivars in the control. In contrast, as a result of water stress, a significant number of flower abortions (10–15%) were observed in cultivar Dundee, LS 677, TGx 1740-2F and TGx 1835-10E which showed the least survival rate at 0%.

The variation observed in control plants however, did not seem to affect pod development and maturation, thus, could be attributed to the genotype performance than the environmental

Soybean genotypes	Mean plant height (cm)	Mean no. of branches	No. of flowering plants (%)	Mean no. of pods produced	Survival frequency (%)
Dundee	31.0 ^a	6.0 ^a	80.0 ^a	21.0 ^a	80.0 ^a
LS 678	41.0 ^b	5.0 ^b	95.0 ^b	32.0 ^b	95.0 ^b
LS 677	49.1 ^c	6.0 ^a	100.0 ^c	36.0 ^c	100.0 ^c
Peking	51.0 ^d	6.0 ^a	100.0 ^c	29.0 ^d	100.0 ^c
TGx 1740-2F	47.1 ^e	5.0 ^b	95.0 ^b	19.0 ^e	95.0 ^b
TGx 1835-10E	49.5 ^c	6.0 ^a	100.0 ^c	21.0 ^a	100.0 ^c

Additional data on yield and yield components of untreated soybean plants

	Ave. pod length (cm)	Ave. pod weight (g)	Seed weight/100 seeds (g)
Dundee	4.06 ^a	0.44 ^a	18.53 ^a
LS 678	3.38 ^b	0.49 ^b	14.06 ^b
LS 677	5.23 ^c	0.50 ^c	14.02 ^b
Peking	3.96 ^d	0.38 ^d	9.54 ^c
TGx 1740-2F	3.40 ^b	0.51 ^c	12.03 ^d
TGx 1835-10E	3.94 ^d	0.49 ^b	12.87 ^e

Plant watering was carried out depending on the moisture availability in the soil. Data on yield components was recorded on the day that the experiment was terminated.

The mean number of pods produced was determined 2 weeks after the pods were successfully produced in order to avoid counting fruit pods that will eventually not produce seeds. Data represent the means and values followed by different letters are significantly different (in columns) (at $p \leq 0.05$) by ANOVA.

Table 3. Vegetative growth and flowering response of soybean plants subjected to normal water conditions.

growth conditions (**Table 3**). In the control, a single genotypic setback was observed in cultivar TGx 1740-2F and TGx 1835-10E, which were the only ones producing the lowest number of pods, respectively. The effect of water stress in other oilseed grains such as sunflower, common bean, wheat, barley and maize were reported [41–43]. According to Jaleel et al. [19] the changes in the photosynthetic pigments and the decrease in metabolic functioning of the plant lead to decreased yield productivity. Seed yield and seed's morphological characters can also be affected by drought [44]. In cultivar Peking, the interaction between water deficit stress and seed appearance resulting from the genotype was not severely pronounced. The seeds appeared intensely shrunk and decreased in seed size due to loss of seed moisture, immediately after harvesting. This response was observed in another study assessing seed longevity in soybean seeds (data not published), clearly suggesting this as a dormancy or viability mechanism compared to other genotypes. In general, significant differences were observed during flowering, pod formation and seed maturation/ filling, as well as in the seed phenotypic characteristics among all cultivars in the control. Many water stressed plants (WT 1 and WT 2) did not survive to reach flowering as observed in the normally irrigated plants of the control (**Tables 2 and 3**).

7. Other biotic and abiotic stress effects

Plants are normally adapted to grow in complex and diverse environments. The success in growth establishment, reproduction and productivity of plant species rely upon a set of environmental conditions, natural resources and the interactions (beneficial or harmful) that exist among plants and other organisms. However, certain types of interactions, especially those including biological factors such as insects, parasites, viruses and bacterial pathogens have detrimental effects on plants. In addition, the non-living physical or chemical factors such as light, temperature, salinity, water, nutrient and other variables that can be found in the aquatic or terrestrial ecosystem also have major impacts on plant life. All above-mentioned factors may induce plant stress, defined by Taiz et al. [17] as a condition that prevent a given plant from achieving its maximum growth and reproductive potential as measured by vegetative growth, flowering, seed formation and yield quantity. Gerhardson [45] gave further information by providing more insights on disease symptoms caused by pathogenic strains of *Fusarium*, *Cylindrocarpon*, *Phoma* and *Pythium* mostly on legume crops. Strains of the genera *Pythium* have also been found to cause seedling mortality in cowpea [46]. These soil-borne legume pathogens, including other wide spread disease causing fungi; induce root, stem and leaf rots in pea, beans and alfalfa [45]. Abiotic environmental stress dramatically affects growth and productivity of many cereals, oilseeds, vegetables and fruit crops.

Oilseeds such as soybeans have suffered major losses from the short and prolonged occurrence of abiotic stress, especially drought, extreme temperatures, flooding and waterlogging [47]. Plants experiencing drought stress may also endure other stress effects simultaneously, like salinity and heat stress. Multiple stress effects and symptoms may be concomitantly induced by the occurrence of a single stress as described by Miransari [48] leading to combinational abiotic stress. In soybean, drought stress has many negative consequences ranging from reduced production of signalling and communication metabolites, decreased photosynthetic assimilates, nutrient deficiency, accumulation of reactive oxygen species (ROS) and reduction in nitrogen (N) fixation by affecting symbiotic relationship with *Rhizobium* species [49–52]. Soybean is an important crop for the production of oils and proteins used for feed and human consumption. It is a potential source for biodiesel and has been used to manufacture a number of pharmaceutical products [53]. But, the high sensitivity to water deficit stress shown in this study by this crop encourage the development of stress tolerant soybean varieties. Drought and other growth constraints are inevitable consequences of climate change. Therefore, investigation on the physiological, anatomical and morphological response of soybean to these biotic and abiotic constraints is highly recommended.

8. Water stress management and crop improvement

As previously discussed, drought stress is the most widely known and devastating stress factor that limit plant growth, development and productivity. Khaine and Woo [54] reported that, frequent drought effects recently and currently experienced, are largely induced by the changes in climatic conditions. The continuously fluctuating meteorological conditions in

many regions worldwide have led to tremendous adversities on agriculture, biodiversity, wildlife and subsequently, the well-beings of many people. Plants normally evolve in order to adapt and adjust to the low water conditions or any other biotic and abiotic constraint. These adaptive measures are an important event of evolution in the history of life, with far reaching consequences as described by Kenrick and Crane [55]. However, this is a very slow process in nature, even if it may result in greater diversity of plants, making changes in plants at their physiological, biochemical and molecular levels. These changes show a wide range of adaptations, at different levels in which plants attempt to deal with drought stress [56]. Plants manage water stress in various ways. They regulate stomatal closure to reduce water loss, especially through transpiration. The stomatal opening and closing is very essential for gaseous exchange as reported by Osakabe et al. [57]. They are controlled by complex regulatory events mediated by abscisic acid (ABA) signalling and ion transport induced by abiotic stress. Nonetheless, stomata closure negatively affects the rates of photosynthetic metabolism by lowering the amount of CO₂. Plants also alter metabolic functions in order to inhibit the production of reactive oxygen species (ROS) such as superoxide (O₂⁻), and H₂O₂ [52, 58]. Other changes involve development of strategies to fix CO₂ with minimum loss of water. For example; many C₃ plants do not have photosynthetic adaptations to reduce the loss of CO₂ molecules by separating photorespiration from the Calvin cycle. However, some succulent plants use Crassulaceae acid metabolism (CAM) to salvage CO₂ minimising photorespiration thus, saving water.

In monocots such as maize and wheat, CO₂ is fixed in the mesophyll spongy cells (a light-dependent process) and in the specialised cell around the leaf veins called the bundle sheath (light-independent). These monocotyledonous plants are referred to as C₄ plants and they produce oxaloacetate which is converted into malate, transported into the bundle sheath for use in Calvin cycle [17, 59, 60]. Both C₄ and CAM plants are well adapted to hot, dry environments than the C₃ plants like peanut (*Arachis hypogaea*), potato (*Solanum tuberosum*) and soybean (*Glycine max* L.). These C₃ plants lack strategies to efficiently and effectively manage water use. In addition to all of the metabolic strategies mentioned above, modern genetic engineering technology can be used. This technology is focused on breeding biotic/ abiotic stress tolerant plants. The biotechnological approaches such as *Agrobacterium*-mediated genetic transformation allow manipulation of the host plant's genome for the expression of foreign genes required in the plant stress response. This technique was initially used to isolate genes used for stress tolerance in *Arabidopsis*. This plant was only used as a model plant and has played an important role in elucidating the basic processes constituting the expression of regulatory genes for stress tolerance [61]. The insights from research on *Arabidopsis* have been used in attempts of unravelling biotic/ abiotic stress effects in plants, subsequently resulting in the development of transgenic plants tolerant to drought, salinity and chilling stress. Montero-Tavera et al. [62] reported upregulation of a number of genes in two common bean varieties with different susceptibility to drought stress. Variety Pinto Villa was relatively susceptible than cultivar Carioca. The reports indicated that drought tolerant variety displayed a more developed root vascular tissue system under stress conditions, when compared to the other non-transgenic cultivars. Differential root phenotype showing variations in root lengths, surface area and fineness of the root system was also reported by Abenavoli et al. [63]. In soybean, stress tolerant genes were introduced and DREB or ARED genes expressed to show improved tolerance to water stress under greenhouse conditions [11, 64]. The genetic

transformation of many crops, including soybean via *in vitro* or *in vivo* transformation techniques is still very difficult to achieve, despite the aforementioned successes. Several drought tolerant cultivars have been reported in rice (*Oryza sativa*), maize (*Zea mays* L.) and kidney bean (*Phaseolus vulgaris* L.) by Liu et al. [65], Saijo et al. [66] and Shou et al. [67]. The methods used for genetic transformation of these crops are continuously optimised to establish efficient and reproducible protocols using *Agrobacterium tumefaciens*. Lastly, agronomic practices such as reduction of water loss from irrigation systems, minimising water inputs and increasing crop water use efficiency can also be employed to manage water stress [68].

9. Conclusions and perspectives

This study revealed that soybeans are primarily affected by water deficit. Cultivars highly susceptible to water stress were easily distinguishable from those showing mild stress effects on the basis of the morphological and anatomical characters in stems, leaves and roots. Morphological architecture, anatomical features and chloroplastidic pigments were significantly affected by the induced water stress. This comprehensive insights regarding the internal and external growth characteristics including, aspects that involve the physiological processes is crucial for the pursuit of genetically modified plants. Soybean remains one of the most important oilseeds that are commercially and subsistently cultivated worldwide. The crop contains higher amounts of proteins, oils, fibre and minerals required for poultry feedstocks and human consumption. As a result of this, the elite superior genotypes of this crop still need to be investigated in order to identify the cultivars that would serve as genetic resource for breeding or genetic engineering, ultimately providing cultivars to be used for agricultural purposes showing high tolerance to abiotic stress, especially drought.

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Author details

Phetole Mangena

Address all correspondence to: mangena.phetole@gmail.com

Department of Biodiversity, School of Molecular and Life Sciences, Faculty of Science and Agriculture, University of Limpopo, Sovenga, South Africa

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