



Effect of waterlogging on growth and development of selected green gram varieties

 Samson Onyango

Ochar¹⁺

Phoebe Anyango

Sikuku²

John Collins Onyango³

^{1,2,3}Department of Botany, Faculty of Biological and Physical sciences, Maseno University, Kisumu-3275-40100, Kenya.

¹Email: ochars2008@gmail.com

²Email: sphoebe@maseno.ac.ke

³Email: jconyango@yahoo.com



(+ Corresponding author)

ABSTRACT

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This experiment studied the effect of waterlogging on growth and development of selected green gram varieties so as to recommend to farmers the variety that can withstand waterlogging. The seeds of the three selected green gram varieties were planted in 10 litre capacity pots that were arranged in completely randomised design. Waterlogging was induced at vegetative stage; 21 days after sowing (DAS) by dipping 10 litre pots in larger 20 litre pots and maintaining standing water at 3cm above the soil level for 3days (*T3*), 6 days (*T6*) and 9 days (*T9*). Control (*T0*) was achieved by watering the pots with 500ml of water per day. Net assimilation rate, transpiration rate and stomatal conductance were measured using LI-COR 680 portable photosynthesis systems. Chlorophyll content was determined using the Arnon method. The measurements were taken from three plant samples per variety regularly throughout the study period. KAT 00301 and KAT 00309 maintained a significantly high net assimilation rate, stomatal conductance and chlorophyll content than KAT 00308 under waterlogging treatments. KAT 00308 was more vulnerable to waterlogging. The variety wilted and died under prolonged waterlogging but KAT 00301 and KAT 00309 survived though with reduced yield. Both small and commercial green gram farmers should plant KAT 00301 and KAT 00309 to minimise losses due to waterlogging.

Contribution/Originality: Green gram being a drought tolerant crop, there is paucity of information on effect of waterlogging on its performance. The study on the effect of waterlogging on growth and development of the newly released varieties by KALRO to recommend the variety that can withstand waterlogging conditions gives the study an originality status.

1. INTRODUCTION

Green gram is the third most important pulse crop after chickpea and pigeon pea. Ecologically, green grams require an altitude of 0-1600m above the sea level, sandy loam and clay soils having a pH range of 5.5-7.5. It is tolerant to drought and has annual rain fall requirement range between 350- 700mm. It has well developed root system with tap root and lateral roots for absorption of water when limited. Being a reliable protein source, green gram is a stable food security source. Mature seeds of green gram contain proteins, carbohydrates, minerals, fibers, as well as antioxidants [1]. Globally, in countries where meat is culturally prohibited from being used as food such as India, green grams offer a reliable protein source [2]. Apart from offering a stable source of food and nutritional security, green gram to many rural households is an important income source. Kanavi, et al. [3] reported that despite the economic importance green grams, abiotic and biotic stress has caused inordinate decline

in its overall production. Green gram has the potential of producing 2.5–3.0 tons per hectare. Despite this potentiality, due to abiotic and biotic constraints, the productivity staggers at 0.5 tons/hectare [4]. Green gram thrives well in Semi-Arid land in Kenya where it is cultivated for both subsistence and commercial purposes. In Machakos, Kitui, Tharaka-Nithi and Makueni counties, green gram production has proved to be more successful and Machakos County leads in green gram production [5]. The newly released varieties of green gram varieties by Kenya agricultural and livestock research organisation (KALRO) include KAT 00301, KAT 00308 and KAT 00309. They are commonly cultivated in most parts of Kenya are All the varieties have short maturity period and perform well in arid and semi-arid lands of Kenya. Table 1 shows the physical characteristics of the selected green gram varieties.

Table 1. Physical characteristics of the newly released green gram varieties.

Green gram variety	Physical properties	Days to maturity	Yield in bags /ha
KAT 00301	<ul style="list-style-type: none"> Grains are green and shiny in color Grain size (6-7g/100 seeds) 	60 - 70	1800-2300kg/ha
KAT 00308	<ul style="list-style-type: none"> Grains are shiny green in color Grain size (8-10g/100 seeds) 	65-75	1800-2100 kg/ha
KAT 00309	<ul style="list-style-type: none"> Grains are green and shiny in color Grain size (8-10g/100 seeds) 	60-65	1800-2100 kg/ha

The early maturity enables them to survive terminal droughts in arid and semi-arid areas. Since green gram is a drought tolerant crop, its performance in waterlogged soils remains questionable. One of the prevalent problem during the growing period of green grams is water logging [6]. The physiological and chlorophyll content responses of the aforementioned green gram varieties to waterlogging is yet to be fully understood. The studies on effect of waterlogging on green grams have been explored and were mostly focused on yield component and a single growth parameter [5, 7-9]. Finding on studies by Ahmed, et al. [9] in particular showed that green grams have the ability to recover from the short-term waterlogging damage and that the response to waterlogging depends on the variety. Amin, et al. [8] reported that survival rate of green gram genotypes was less than 20%. Kumar, et al. [10] reported that green gram varieties subjected to waterlogging for nine days lost photosynthetic apparatus by up to more than 80% and did not recover from waterlogging damage. The above conclusions were arrived at by analysing at least one physiological parameter. Since physiological parameters and chlorophyll development directly influence morphology and yield, it is important to investigate further on how waterlogging affects the physiological parameters and chlorophyll content of the newly released varieties of green grams particularly in Kenya where such studies have remained relatively scanty.

More than 90% of agricultural systems in Kenya are rain-fed thus highly vulnerable to changes in climate. There is an overall shift in the rainfall distribution, with floods becoming more likely than the opposite extreme [11]. The rainfall pattern changes in Kenya have been attributed to climate change due to global warming. Rainfall fluctuations due to erratic and unpredictable rainfall patterns exposes the rain fed agricultural systems in Kenya to more vulnerabilities. Prolonged flooding affects physiological parameters and chlorophyll content of green grams. The physiological and chlorophyll content response to waterlogging is manifested in morphology and yield components of green grams. A shift in water regimes during early growth stages destabilises the crop growth and negatively affects the subsequent stages, which in turn affects the final yield. It is therefore necessary to assess the physiological and chlorophyll content response of selected green gram varieties to water logging and determine the varieties that can withstand waterlogging. The study was therefore conducted to determine the effect of waterlogging on transpiration rate, stomatal conductance, net assimilation rate and chlorophyll content of selected green gram varieties to determine the variety that can withstand waterlogging with an aim of recommending the variety to farmers as well as to plant breeders for further breeding work towards water logging tolerance.

2. MATERIALS AND METHODS

2.1. Experimental Site and Climate

The research was carried out in the University Botanic Garden, Maseno-Kenya under greenhouse conditions. The University is located in western region of Kenya; along the equator. The climate is hot and humid with temperatures ranging from 66°F to 83°F.

2.2. Experimental Design and Treatments

The experiment was carried out in a complete randomised design with four treatments each having three replications. The treatments were as follows:

T0- control.

T3- Three days water logging.

T6- Six days water logging.

T9- Nine days water logging.

2.3. Experimental Lay Out

The research experimental unit constituted a ten-litre capacity pot. The pots were perforated and filled with solarised soil collected from the University Botanic Garden. The soil was collected by use of a jembe and soil auger and was mixed with two teaspoonful of DAP fertilizer per pot to hasten root establishment. The seeds of the three varieties; KAT 00301, KAT 00308 and KAT 00309 were obtained from KALRO-Kisumu.

2.4. Sowing and Crop Management

Ten seeds of each variety were planted per pot and thinning to three was done fourteen days after sowing. From the day of sowing, each pot was watered with 500 ml of water per day. At 21 DAS, waterlogging was induced for three successive days for treatment one (T3), six successive days for treatment two (T6) and nine successive days for treatment three (T9). The treatments were modified from Amin, et al. [12]. The water logging status was achieved by dipping the 10 litre pots containing green gram crops into larger 20 litre pots and afterwards, standing water at 3 cm above the soil surface was maintained in each pot for the specified number of days in each treatment. This was modified from Amin, et al. [12] and Ahmed, et al. [9]. Each treatment was replicated three times and the pots were arranged in a Completely Randomised Design (CRD). This was accordance with Amin, et al. [12]. Control treatment was achieved by watering each pot with 500ml of water per day throughout the growing period of the green grams. After termination of waterlogging, the 20 litre pots were removed and each pot watered with 500ml of water per day throughout the growing period. This was modified from Sosiawan, et al. [13]. Weed control was achieved by uprooting them from the pots.

2.5. Data Collection

The data on net assimilation rate, transpiration rate and stomatal conductance were measured using LI-COR 680 portable photosynthesis systems and chlorophyll content was determined using the Arnon method. The data was taken from three plant samples of each variety for every treatment and throughout the study period after initiating the treatments.

2.6. Data Analysis

The data were subjected to analysis of variance using SAS (version 9.1) and separation done using LSD test at 5% level.

3. RESULTS

3.1. Net Assimilation Rate

Three days, six days and nine days waterlogging significantly reduced the net assimilation rate of all the three green gram varieties ($p \leq 0.05$). At three days waterlogging, there was a significant difference in net assimilation rate among the three varieties ($p \leq 0.05$) with KAT 00309 maintaining a higher rate of net assimilation as compared to KAT 00301 and KAT 00308. At six-days waterlogging, there was no significant difference in reduction in net assimilation rate among the three varieties ($p \geq 0.05$). At nine days water logging regime, KAT 00301 maintained significantly higher net assimilation rate as compared to KAT 00309 ($p \geq 0.05$). Table 2 shows the effect of different waterlogging regimes on net assimilation rate ($\mu\text{mol-2s}^{-1}$) of the three green gram varieties. The letters a, b and c indicate significant difference between the varieties for each treatment. Means with the same letter for a particular treatment are not statistically significantly different.

Table 2. Effect of different waterlogging regimes on net assimilation rate ($\mu\text{mol-2s}^{-1}$) of the three green gram varieties.

	<i>T0</i>	<i>T3</i>	<i>T6</i>	<i>T9</i>
<i>KAT 00309</i>	19.336a	6.1371a	0.5394a	0.5166a
<i>KAT 00308</i>	8.981b	2.4318b	0.4592a	0.0000c
<i>KAT 00301</i>	9.484b	0.5039c	0.44084a	1.6048b
<i>LSD</i>	3.6989	1.3911	0.4445	0.3977

Note: Means with the same letters along a column are not significantly different ($p \geq 0.05$). The values represent the means of three replicates.

3.2. Stomatal Conductance

Waterlogging significantly reduced stomatal conductance of all the green gram varieties ($p \leq 0.05$). At three days and six days waterlogging regimes, KAT 00309 and KAT 00301 had higher rate of stomatal conductance than KAT 00308, however, rates of stomatal conductance among the three varieties were not significantly different ($p \geq 0.05$). At nine days water logging regime, KAT 00301 had significantly lower rate of stomatal conductance than KAT 00309 ($p \leq 0.05$). At six days and nine days water logging regime, all the KAT 00308 variety plants had withered. Table 3 presents the effect of waterlogging on stomatal conductance (mol-2s^{-1}) of the three green gram varieties. The letters a, b and c indicate significant difference means between the varieties for each treatment. Means with the same letter for a particular treatment are not statistically significantly different.

Table 3. Effect of different water logging regimes on stomatal conductance (mol-2s^{-1}) of the three green gram varieties.

	<i>T0</i>	<i>T3</i>	<i>T6</i>	<i>T9</i>
<i>KAT 00309</i>	0.69840a	0.05498a	0.05263a	0.06793c
<i>KAT 00308</i>	0.12557b	0.02186a	0.01476a	0.00000b
<i>KAT 00301</i>	0.51158a	0.05342a	0.04039a	0.032302a
<i>LSD</i>	0.206	0.0415	0.0512	0.0229

Note: Means with the same letters along a column are not significantly different ($p \geq 0.05$). The values represent the means of three replicates.

3.3. Transpiration Rate

Waterlogging significantly reduced the rate of transpiration in all green gram varieties with nine days having the greatest impact ($p \leq 0.05$). At three days waterlogging and six days waterlogging, there was a significant difference in reduction in transpiration rate between KAT 301 and the other two varieties ($p \leq 0.05$). At nine days water logging, there was no significant difference in reduction in transpiration rate between KAT 00301 and KAT 00309 ($p \geq 0.05$). At six days and nine days water logging regimes, all the KAT 00308 plants withered. Table 4 presents the effect of waterlogging on transpiration rate (mol-2s^{-1}) of the three green gram varieties. The letters a, b and c indicate significant difference between the varieties for each treatment. Means with the same letter for a particular treatment are not statistically significantly different.

Table 4. Effect of different water logging regimes on transpiration rate(mol-2s-1) of the three green gram varieties.

	<i>T0</i>	<i>T3</i>	<i>T6</i>	<i>T9</i>
<i>KAT 00309</i>	0.0169621a	0.003835b	0.0006570b	0.0005685a
<i>KAT 00308</i>	0.0070334b	0.001411b	0.0004608b	0.000000b
<i>KAT 00301</i>	0.0143091c	0.004026a	0.0034330a	0.0006357a
<i>LSD</i>	0.0018	0.0026	0.0015	0.0005

Note: Means with the same letters along a column are not significantly different ($p \geq 0.05$). The values represent the means of three replicates.

3.4. Chlorophyll Content

Waterlogging significantly reduced the chlorophyll content in all green gram varieties ($p \leq 0.05$). At three days waterlogging, KAT 00309 had significantly higher chlorophyll content as compared to KAT 00308 and 00301 ($p \leq 0.05$). At six days waterlogging regime, chlorophyll content among the three green gram varieties was not significantly different ($p \geq 0.05$) however, KAT 00309 recorded a higher chlorophyll content compared to the other varieties. At nine days waterlogging regime, KAT 00301 had significantly higher chlorophyll content than KAT 00309 ($p \leq 0.05$). At this treatment, KAT 00308 had withered and died. Table 5 presents the effect of waterlogging on chlorophyll content(mol-2s-1) of the three green gram varieties. The letters a, b and c indicate significant difference between the varieties for each treatment. Means with the same letter for a particular treatment are not statistically significantly different.

Table 5. Effect of different water logging regimes on chlorophyll content of the three green gram varieties (mg m-2).

	<i>T0</i>	<i>T3</i>	<i>T6</i>	<i>T9</i>
<i>KAT 00309</i>	19.336a	6.1371a	0.5394a	0.5166 b
<i>KAT 00308</i>	8.981b	2.4318 b	0.4592a	0.0000b
<i>KAT 00301</i>	9.484b	0.5039 c	0.4408a	1.6048a
<i>LSD</i>	3.6989	1.3911	0.4445	0.6288

Note: Means with the same letters along a column are not significantly different ($p \geq 0.05$). The values represent the means of three replicates.

4. DISCUSSION

4.1. Net Assimilation Rate

The rate of net assimilation in all green gram varieties significantly reduced under different waterlogging conditions ($p \leq 0.05$). This is in agreement with findings of Prasanna and Ramarao [14] who reported that four- and six-day waterlogging significantly lowered net assimilation rate in pigeon pea genotypes. However, the results are not consistent with findings of Oo, et al. [15] who reported that the net assimilation rate in green gram genotypes was less affected by flooding. Reduction in net assimilation rate is attributed to reduced stomatal conductance and reduction in leaf area. Reduced stomatal conductance reduces the rate of diffusion of carbon dioxide into the mesophyll cells thus lowered carbon dioxide assimilation. Lowered net assimilation rate could also be linked to chlorosis which leads to loss of photosynthetic pigment hence reduced surface area for photosynthesis. Increase in duration of waterlogging increased chlorosis in all green gram varieties. According to Islam, et al. [16] reduction in photosynthetic activity in green gram genotypes under water logging conditions is due to both stomatal and non-stomatal limitations.

The study revealed that KAT 00308 was more vulnerable to waterlogging hence had the lowest rate of carbon dioxide assimilation. KAT 00301 and KAT 00309 maintained significantly slightly higher rate of carbon dioxide assimilation under waterlogging stress. KAT 00308 lacks mechanisms to counter chlorophyll degradation hence experienced rapid chlorophyll degradation that greatly limited photosynthetic activities. Moreover, KAT 00301 and KAT 00309 developed mechanisms to counter rapid chlorophyll degradation hence photosynthetic activities were not greatly affected.

4.2. Stomatal Conductance

Waterlogging significantly reduced stomatal conductance in all three green gram varieties. This confirmed the findings of Islam, et al. [16] and Takele and McDavid [17]. Reduction in stomatal conductance under waterlogging stress is a mechanism employed by plants to curtail transpiration. This is supported by the fact that waterlogging stress in all green gram varieties under study significantly lowered the rate of transpiration. Waterlogging may have led to accumulation of ABA which induced stomatal closure hence increased stomatal resistance. High stomatal conductance resistance may also be attributed to lack of oxygen availability in the soil around the root hairs which tampers with normal functioning of roots.

Among the three green gram varieties, there was a significant difference in stomatal conductance at different waterlogging conditions. KAT 00308 had significantly lower rate of stomatal conductance as compared to KAT 00301 and KAT 00309. This showed that KAT 00308 is highly vulnerable to water logging. Significantly higher stomatal conductance in KAT 00310 and KAT 00309 green gram varieties showed that the two varieties may withstand waterlogging due to possession of mechanisms that counter stomatal conductance.

4.3. Transpiration Rate

The rate of transpiration significantly reduced in all green gram varieties with increase in days of waterlogging ($p \leq 0.05$). Reduction in the rate of transpiration with increase in duration of waterlogging is linked to increase in stomatal closure. This is in agreement with findings of Worku [18] who reported that increase in water logging duration significantly decreased the rate of transpiration in green grams. Takele and McDavid [17] also reported that an increase in water logging period decreased the rate of transpiration in pigeon pea genotypes while Ahmed, et al. [9] reported that 8 day water logging sharply declined the rate of transpiration in mung bean genotypes.

There was a significant difference in transpiration rate among the three green gram varieties. KAT 03008 had significantly lower transpiration rate, as compared to KAT 00301 and KAT 00309 at waterlogging treatments. Significantly low rate of transpiration under water logging in KAT 00301 is attributed to rapid stomatal closure. Similarly, significantly higher rate of transpiration in KAT 00301 and KAT 00309 under water logging could be attributed to reduced stomatal closure.

4.4. Chlorophyll Content

Waterlogging significantly reduced chlorophyll content in all green gram varieties. The reduction was more pronounced with increase in duration of waterlogging. This is in agreement with findings of Prasanna and Ramarao [14] in green grams and Kumar, et al. [10] in green grams. Decrease in chlorophyll content may be attributed to chlorophyll degradation and chlorosis. Decrease in chlorophyll content may also be linked to decrease in nitrogen content of leaves due to cessation of biological nitrogen fixation as a result of water logging.

Among the three green gram varieties under study, KAT 00308 showed significantly low chlorophyll content as compared to KAT 00301 and KAT 00309 under water logging treatments. Loss of chlorophyll was evidenced by increased yellowing of leaves which was eventually followed by wilting and death of KAT 00308 under six days and nine days waterlogging regimes. Faster chlorophyll degradation in KAT 00308 may be attributed to almost immediate cessation of nitrogen uptake under waterlogging conditions. Significantly higher chlorophyll content in KAT 00301 and KAT 00309 under waterlogging conditions may suggest that nitrogen uptake in the varieties is not greatly affected by water logging.

5. CONCLUSION

Notably, KAT 00308 maintained significantly relatively low net assimilation rate, low rate of transpiration and low stomatal conductance under waterlogging regimes as compared to KAT 00301 and KAT 00309. Similarly, KAT 00308 showed faster chlorosis while KAT 00301 and KAT 00309 maintained a higher chlorophyll content

under waterlogging regimes. Six day and nine-day waterlogging were detrimental to KAT 00308 hence all wilted and died, failing to reach reproductive stage. The variety therefore cannot withstand prolonged flooding conditions.

KAT 0301 and KAT 309 survived six- and nine-day waterlogging though with reduced final yield. The two varieties showed some degree of tolerance to waterlogging with KAT 00309 less affected physiologically. Both small and commercial green gram farmers should plant either KAT 00301 or KAT 00309 to minimise losses due to waterlogging. Moreover, either KAT 00301 or KAT 00309 could therefore a better variety to be improved through genetic experiments to varieties that can withstand waterlogging for continued green gram production throughout all seasons.

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