



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

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### ABSTRACT

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This experiment studied the effect of waterlogging on the growth and development of selected green gram varieties to recommend the variety that can withstand waterlogging. The seeds of three selected green gram varieties were planted in 10-litre capacity pots arranged in a completely randomized design. Waterlogging was induced at the vegetative stage, 21 days after sowing (DAS), by dipping 10-litre pots into larger 20-litre pots and maintaining standing water at 3cm above the soil level for 3 days ( $T_3$ ), 6 days ( $T_6$ ), and 9 days ( $T_9$ ). The control ( $T_0$ ) 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. Measurements were taken from three plant samples per variety regularly throughout the study period. KAT 00301 and KAT 00309 maintained significantly higher net assimilation rates, 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 minimize losses due to waterlogging.

**Contribution/Originality:** Green gram, being a drought-tolerant crop, has limited information regarding the effects of waterlogging on its performance. The study on the impact of waterlogging on the growth and development of newly released varieties by KALRO aims to identify varieties that can withstand waterlogging conditions, thereby giving 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 sea level, sandy loam and clay soils with a pH range of 5.5-7.5. It is tolerant to drought and has an annual rainfall requirement range between 350-700mm. It has a well-developed root system with tap roots and lateral roots for water absorption 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 source of protein [2]. Apart from offering a stable source of food and nutritional security, green gram is an important income source for many rural households. Kanavi et al. [3] reported that despite the economic importance of green grams, abiotic and biotic stresses have caused an inordinate decline in its overall

production. Green gram has the potential to produce 2.5–3.0 tons per hectare. Despite this potential, due to abiotic and biotic constraints, the productivity stagnates 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 proven to be more successful, with Machakos County leading in green gram production [5]. The newly released varieties of green gram by the Kenya Agricultural and Livestock Research Organization (KALRO) include KAT 00301, KAT 00308, and KAT 00309. They are commonly cultivated in most parts of Kenya. All the varieties have a short maturity period and perform well in the 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"> <li>Grains are green and shiny in color</li> <li>Grain size (6-7g/100 seeds)</li> </ul>	60 - 70	1800-2300kg/ha
KAT 00308	<ul style="list-style-type: none"> <li>Grains are shiny green in color</li> <li>Grain size (8-10g/100 seeds)</li> </ul>	65-75	1800-2100 kg/ha
KAT 00309	<ul style="list-style-type: none"> <li>Grains are green and shiny in color</li> <li>Grain size (8-10g/100 seeds)</li> </ul>	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 problems during the growing period of green grams is waterlogging [6]. The physiological and chlorophyll content responses of the aforementioned green gram varieties to waterlogging are yet to be fully understood. Studies on the effect of waterlogging on green grams have been explored, and most of these studies focused on yield components and a single growth parameter [5, 7-9]. According to studies by Ahmed et al. [9], in particular, it was shown that green grams have the ability to recover from short-term waterlogging damage, and that the response to waterlogging depends on the variety. Amin et al. [8] reported that the 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 their photosynthetic apparatus by more than 80% and did not recover from waterlogging damage. The above conclusions were arrived at by analyzing at least one physiological parameter. Since physiological parameters and chlorophyll development directly influence morphology and yield, it is important to investigate further 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, they are highly vulnerable to changes in climate. There is an overall shift in rainfall distribution, with floods becoming more likely than the opposite extreme [11]. The rainfall patterns in Kenya have been changing, which has been attributed to climate change caused by global warming. Fluctuations in rainfall due to unpredictable and erratic patterns expose rain-fed agricultural systems in Kenya to increased vulnerabilities. Prolonged flooding impacts the physiological parameters and chlorophyll content of green grams. The response of physiological and chlorophyll content to waterlogging manifests in the morphology and yield components of green grams. A shift in water regimes during early growth stages destabilizes crop development and negatively influences subsequent stages, ultimately affecting final yield. Therefore, it is essential to assess the physiological and chlorophyll content responses of selected green gram varieties to waterlogging and identify varieties capable of withstanding such conditions. The study was conducted to determine the effects of waterlogging on transpiration rate, stomatal conductance, net assimilation rate, and chlorophyll content of selected green gram varieties, with the aim of recommending suitable varieties to farmers and plant breeders for further breeding efforts towards waterlogging tolerance.

## 2. MATERIALS AND METHODS

### 2.1. Experimental Site and Climate

The research was conducted at the University Botanic Garden in Maseno, Kenya, under greenhouse conditions. The university is located in the 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 conducted using a completely randomized design with four treatments, each having three replications. The treatments were as follows:

T0- control.

T3- Three days of water logging.

T6- Six days of water logging.

T9- Nine days of water logging.

### 2.3. Experimental Layout

The research experimental unit consisted of a ten-litre capacity pot. The pots were perforated and filled with solarized soil collected from the University Botanic Garden. The soil was collected using a jembe and soil auger and was mixed with two teaspoonfuls 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 performed fourteen days after sowing. From the day of sowing, each pot was watered with 500 ml of water daily. 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 waterlogging status was achieved by immersing 10-litre pots containing green gram crops into larger 20-litre pots, and subsequently maintaining standing water at 3 cm above the soil surface in each pot for the specified number of days in each treatment. This method 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 randomized design (CRD). This was in accordance with [Amin et al. \[12\]](#). Control treatment was achieved by watering each pot with 500 ml of water per day throughout the growing period of the green grams. After the termination of waterlogging, the 20-litre pots were removed, and each pot was watered with 500 ml 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 were collected from three plant samples of each variety for every treatment 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 was performed using the LSD test at a 5% significance level.

### 3. RESULTS

#### 3.1. Net Assimilation Rate

Three days, six days, and nine days of waterlogging significantly reduced the net assimilation rate of all three green gram varieties ( $p \leq 0.05$ ). At three days of waterlogging, there was a significant difference in the net assimilation rate among the three varieties ( $p \leq 0.05$ ), with KAT 00309 maintaining a higher rate of net assimilation compared to KAT 00301 and KAT 00308. At six days of waterlogging, there was no significant difference in the reduction of the net assimilation rate among the three varieties ( $p \geq 0.05$ ). At nine days of waterlogging, KAT 00301 maintained a significantly higher net assimilation rate compared to KAT 00309 ( $p \leq 0.05$ ). Table 2 shows the effect of different waterlogging regimes on the net assimilation rate ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) of three green gram varieties. The letters a, b, and c indicate significant differences 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 ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) 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 and six days of waterlogging regimes, KAT 00309 and KAT 00301 exhibited higher rates of stomatal conductance than KAT 00308; however, the rates of stomatal conductance among the three varieties were not significantly different ( $p \geq 0.05$ ). At nine days of waterlogging regime, KAT 00301 had a significantly lower rate of stomatal conductance than KAT 00309 ( $p \leq 0.05$ ). During the six and nine days waterlogging regimes, all plants of the KAT 00308 variety had withered. Table 3 presents the effect of waterlogging on stomatal conductance ( $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) of the three green gram varieties. The letters a, b, and c indicate significant differences between the means of 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 ( $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) 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 of waterlogging and six days of waterlogging, there was a significant difference in the reduction of transpiration rate between KAT 301 and the other two varieties ( $p \leq 0.05$ ). At nine days of waterlogging, there was no significant difference in the reduction of transpiration rate between KAT 00301 and KAT 00309 ( $p \geq 0.05$ ). At six days and nine days of waterlogging regimes, all the KAT 00308 plants withered. Table 4 presents the effect of waterlogging on the transpiration rate ( $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) of the three green gram varieties. The letters a, b, and c indicate significant differences 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 of waterlogging, KAT 00309 had a significantly higher chlorophyll content compared to KAT 00308 and KAT 00301 ( $p \leq 0.05$ ). At six days of waterlogging, 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 of waterlogging, KAT 00301 had a significantly higher chlorophyll content than KAT 00309 ( $p \leq 0.05$ ). At this stage, 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 differences 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 decreased under different waterlogging conditions ( $p \leq 0.05$ ). This is consistent with previous 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 lowering carbon dioxide assimilation. Lowered net assimilation rate could also be linked to chlorosis, which leads to loss of photosynthetic pigment, hence reducing the surface area for photosynthesis. An increase in the duration of waterlogging increased chlorosis in all green gram varieties. According to Islam et al. [16] deduction in photosynthetic activity in green gram genotypes under waterlogging conditions is due to both stomatal and non-stomatal limitations.

The study revealed that KAT 00308 was more vulnerable to waterlogging, hence it had the lowest rate of carbon dioxide assimilation. KAT 00301 and KAT 00309 maintained slightly higher rates of carbon dioxide assimilation under waterlogging stress. KAT 00308 lacks mechanisms to counter chlorophyll degradation, hence it experienced rapid chlorophyll degradation that greatly limited photosynthetic activities. Moreover, KAT 00301 and KAT 00309 developed mechanisms to counter rapid chlorophyll degradation; therefore, 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 the accumulation of ABA, which induced stomatal closure, hence increasing stomatal resistance. High stomatal conductance resistance may also be attributed to the lack of oxygen availability in the soil around the root hairs, which hampers the normal functioning of roots.

Among the three green gram varieties, there was a significant difference in stomatal conductance under different waterlogging conditions. KAT 00308 had a significantly lower rate of stomatal conductance compared to KAT 00301 and KAT 00309. This indicates that KAT 00308 is highly vulnerable to waterlogging. Significantly higher stomatal conductance in KAT 00310 and KAT 00309 suggests that these two varieties may withstand waterlogging due to mechanisms that regulate stomatal conductance.

#### 4.3. Transpiration Rate

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

There was a significant difference in transpiration rates among the three green gram varieties. KAT 03008 had a significantly lower transpiration rate compared to KAT 00301 and KAT 00309 under waterlogging treatments. The significantly low rate of transpiration in KAT 00301 under waterlogging is attributed to rapid stomatal closure. Similarly, the higher rates of transpiration in KAT 00301 and KAT 00309 under waterlogging 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 an increase in the duration of waterlogging. This is in agreement with the findings of Prasanna and Ramarao [14] in green grams and Kumar et al. [10] in green grams; a decrease in chlorophyll content may be attributed to chlorophyll degradation and chlorosis. A reduction in chlorophyll content may also be linked to a decrease in nitrogen content of leaves due to the cessation of biological nitrogen fixation as a result of waterlogging.

Among the three green gram varieties studied, KAT 00308 exhibited significantly lower chlorophyll content compared to KAT 00301 and KAT 00309 under waterlogging treatments. The loss of chlorophyll was evidenced by increased yellowing of leaves, which was eventually followed by wilting and death of KAT 00308 under six- and nine-day waterlogging regimes. Faster chlorophyll degradation in KAT 00308 may be attributed to the almost immediate cessation of nitrogen uptake under waterlogging conditions. Significantly higher chlorophyll content in KAT 00301 and KAT 00309 under waterlogging conditions suggests that nitrogen uptake in these varieties is not greatly affected by waterlogging.

### 5. CONCLUSION

Notably, KAT 00308 maintained a relatively low net assimilation rate, low transpiration rate, and low stomatal conductance under waterlogging regimes compared to KAT 00301 and KAT 00309. Similarly, KAT 00308 exhibited



faster chlorosis, while KAT 00301 and KAT 00309 maintained higher chlorophyll content under waterlogging regimes. Six-day and nine-day waterlogging treatments were detrimental to KAT 00308; hence, all plants wilted and died, failing to reach the 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 minimize losses due to waterlogging. Moreover, either KAT 00301 or KAT 00309 could therefore be better varieties to be improved through genetic experiments to develop varieties that can withstand waterlogging for continued green gram production throughout all seasons.

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