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Effect of Arbuscular mycorrhizal inoculation on growth, biochemical characteristics and nutrient uptake of passion fruit seedlings under flooding stress

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Abstract

This study was undertaken to investigate the role of arbuscular mycorrhiza in alleviation of flooding stress in passion fruits in Kenya. Passion fruit seedlings (*Passiflora edulis* var edulis L.) (purple passion fruits) were raised in sterilized sand under low phosphorus regime for 12 weeks before flooding was initiated for 28 days. Mycorrhizal inoculation maintained greater leaf retention as opposed to leaf abscission that occurred more rapidly in non-mycorrhizal seedlings under flooding. Flooding induced an increase in leaf proline concentration with mycorrhizal seedlings having the highest proline concentration. Flooding caused a decline in chlorophyll content and this occurred more rapidly in non mycorrhizal treatments. Flooding also caused an increase in the carotenoid content, this occurring more rapidly in nonmycorrhizal seedlings. The total soluble sugars increased in non-mycorrhizal seedlings subjected to flooding but remained unchanged in mycorrhizal seedlings under flooding. Flooding induced a reduction but did not completely inhibit mycorrhizal root colonization. The leaf nitrogen and phosphorus content declined under flooding, with the decline occurring more rapidly in non-mycorrhizal seedlings. This study found out that increased production of proline, maintenance of optimum nutrient supply in the leaves and maintenance of leaf chlorophyll aid mycorrhizal passion fruit seedlings to delay the adverse effects of flooding.

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Introduction

Flooding is one of the weather phenomena that affect many regions of the world. On a world scale, the land area exposed to flooding is > 17 million km² (Perata *et al.* 2011). Kenya was ranked among the 16 worst affected tropical countries during the 1997/98 El Niño event which resulted in severe floods after major rivers in the country attained record peaks thereby causing havoc and destroying livelihoods (Gichere *et al.*, 2013). Studies on the role of arbuscular mycorrhiza on flooding stress tolerance have been limited, and have mainly been confined to flood tolerant crop species such as rice and other plant species such as mangroves (Parlanti *et al.,* 2011). A few studies have however been undertaken in flood-intolerant crops. Study in Ullapara, Bangladesh, found abundant AM spores in flooded farmers' fields (Khanam, 2008). Heavy colonization was subsequently observed in onion roots grown after the flood water subsided (Khanam, 2008). Flooded mycorrhizal seedlings accumulated higher proline than non-mycorrhizal seedlings in *Aster tripolium* study in Portugal (Neto *et al.,* 2006). Total chlorophyll content decreased under flooding with mycorrhizal plants maintaining higher chlorophyll than non mycorrhizal plants (Hajiboland *et al.,* 2009). Studies in maize in Iran showed that the amount of soluble sugars increased 1.5-2 times during the early stage of flooding (Pourabdal *et al.,* 2008). However, increasing the flooding period decreased this ratio and the amount of sugars gradually decreased and finally reached the control levels (Pourabdal *et al.,* 2008).

Passion fruit is one of the most important fruit crop in Kenya both for local consumption and for export (HCDA, 2012). Unfortunately, there has been no study undertaken to determine the effect of AM fungi on passion fruit seedlings growth under flooding conditions. This study aims to address this problem.

Materials and methods

The study was undertaken in Jomo Kenyatta University of Agriculture and Technology (JKUAT) in Kiambu County (1255 m asl, 1.03°S, 37.01°E). Passion fruit (*Passiflora edulis* var edulis (purple) seeds were germinated in sterile sand and uniform seedlings selected and transplanted to 5 liter polythene pots (20 cm in diameter and 25cm depth) filled with sterilized sterile sand media (1:1 vol/vol) in a polyethylene-covered greenhouse. At transplanting, seedlings were inoculated with 50 grams of AM inoculum containing approximately 200 spores of a mixture of *Glomus caledonium, G. etunicatum, Gigaspora magarita* and *Scutellospora sp* (Plantworks Inc., UK). To ensure uniformity, similar quantities of autoclaved inoculum were added to the non-mycorrhizal pots.

The experiment was laid out in Completely Randomized Design consisting of 20 seedlings per treatment (mycorrhizal and non mycorrhizal) subjected to flooding and a similar number of seedlings used as non-flooded controls. Seedlings were raised for 12 weeks before flooding was initiated and were watered weekly with 300 mls of Hoagland's nutrient solution modified by halving the phosphorus content (Table 1). The flooding experiment was set up by placing the potted seedlings in wide, non-perforated wooden structures supported by polythene to hold the water (Plate 1). Water was regularly piped into the structure so that the media was covered by water to about 2cm above the surface. This water level was maintained throughout the flooding period of 28 days before the experiment was terminated.

At the start of flooding and every 7 days, weekly measurements were taken on plant leaf number, chlorophyll and carotenoid contents, proline and total soluble sugars (TSS), mycorrhizal root colonisation and leaf nitrogen and phosphorus levels.

Evaluation of root colonization levels

At seedling harvest, root tips (1 ± 0.2cm) were excised and cleared by autoclaving in 10% KOH followed by staining in 0.05% tryphan blue, glycerol and lactic acid (1:1:1) solution. The frequency of mycorrhizal colonization was noted per field (10 grids) for 10 fields, using the grid intersect method (Giovannetti and Mosse, 1980). To convert the data into percent colonization, the frequency of colonization as a fraction of the total number of grids observed was multiplied by 100.

Nutrient analysis

Oven-dried leaves were ground with a mortar and pestle and 1 gram weighed and dry-ashed by heating for 5 hours at 550°C in a muffle furnace. The ash was taken up in 20% HCl and the solution made up to 20 mls with distilled deionised water. Two hundred microliter aliquots from these solutions were further diluted to 10 mls. Phosphorus, as molybdate-reactive P was measured by blue colorimetry at 730 nm using a spectrophotometer (Ref). The nitrogen estimation was done by micro Kjeldahl method (Ref).

Chlorophyll and carotenoids determination

The chlorophyll a and b were determined according to the methods of Arnon (1949) and carotenoids according to Davies (1976). The fresh leaves were cut to 0.5cm segments and extracted overnight in 80% acetone at -10°C. The extract was centrifuged at 14000 x *g* for 5 minutes and the absorbance of the supernatant was read at 480, 645 and 663 nm using a spectrophotometer. The chlorophyll a, b and the total chlorophyll and carotenoids was calculated using the formula below:

Chl a = [12.7 (OD 63 – 2.69 (od 645)] x V/1000 x W

Chl b = [22.9 (OD 645 – 4.68 (od 663)] x V/1000 x W

Total Chlorophyll = Chl a + chl b

V = volume of the extract (mls)

W = weight of the fresh leaf tissue (grams)

Carotenoids $gml^{-1} = A^{car}/Em \times 100$

Where A^{car} = OD 480 + 0.14 (od 663) – 0.638 (OD 645)

$E^{100\%}cm = 2500$

Proline and total soluble sugars

Free proline and total soluble sugars were extracted from 1g of fresh roots and leaves. Proline was estimated by spectrophotometric analysis at 515nm of the ninhydrin reaction, according to Bates *et al.* (1973). Soluble sugars were analyzed by 0.1 ml of the alcoholic extract reacting with 3ml freshly prepared anthrone (200 mg anthrone + 100ml 72% (w:w) H_2SO_4) and placed in a boiling water bath for 10 min according to Irigoyen *et al.* (1992). After cooling, the absorbance at 620nm was determined in a spectrophotometer. The calibration curve was made using glucose in the range of 20–400µg ml^{-1}.

Statistical analysis

The data obtained was subjected to ANOVA, using Genstat software (2013). All treatment means were tested for LSD and contrasts between means made using the Genstat software (2013).

Results

Leaf number

Leaf growth (as measured by increase in leaf number) continued in unflooded treatments but declined in treatments subjected to flooding (Fig 1). The decline occurred in non-mycorrhizal seedlings by 14th day of flooding due to leaf abscission while in mycorrhizal seedlings, it occurred after 21 days (Fig. 1).

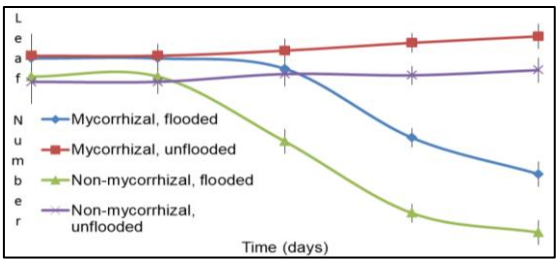

Fig. 1. Effect of AM fungi and flooding on the leaf number of passion fruit seedlings

Proline concentration

The proline concentration was low at the start of flooding and remained constantly low in unflooded treatments (Fig. 2). It increased in flooded treatments from the 14th day, but decreased to the unflooded levels by the 28th day (Fig. 2). The highest proline concentration was achieved by flooded, mycorrhizal seedlings (Fig. 2). The proline concentration peaked in flooded mycorrhizal seedlings on the 14th day, while in flooded non-mycorrhizal seedlings, the peak occurred just before the 21st day (Fig. 2).

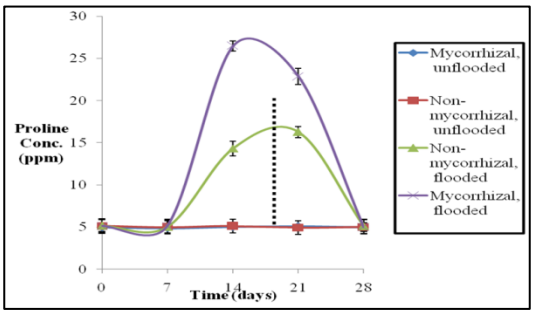

Fig. 2. Feecet of AM fungi and flooding on the Proline concentration (ppm) of passion fruit seedlings.

Chlorophyll and Carotenoids content

The chlorophyll contents remained unchanged in unflooded treatments but declined under flooding (Fig. 3). The total chlorophyll levels were significantly lower under 7, 14 and 21 days of flooding in non-mycorrhizal treatments compared to flooded mycorrhizal treatments but by the 28th day, there was no significant difference in the levels between the two treatments ((Fig. 3). The unflooded treatments maintained low carotenoid content while the levels increased under flooding (Fig. 4). Under 7, 14 and 21 day of flooding, the carotenoid level was significantly higher in non-mycorrhizal seedlings under flooding but the levels were similar after 28 days of flooding (Fig. 4).

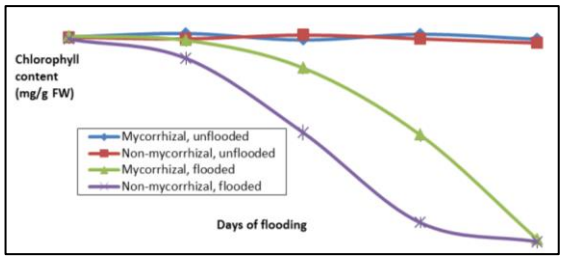

Fig. 3. Effect of AM fungi and flooding on the Total Chlorophyll of passion fruit seedlings.

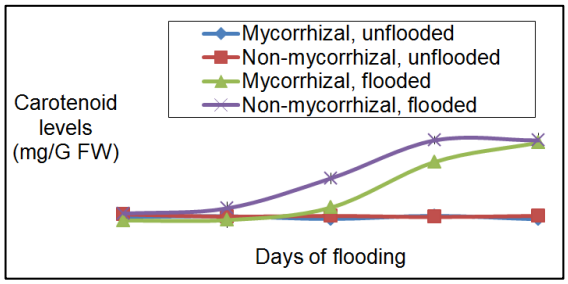

Fig. 4. Effect of AM fungi and flooding on the Total Carotenoid levels of passion fruit seedlings.

Mycorrhizal root colonization

Mycorrhizal root colonization remained constant under unflooded conditions (Table 2.0). Under flooding, the colonization declined, starting from the 14th day, but was not completely inhibited (Table 2.0).

Soluble sugar content

The leaf and root soluble sugar content remained constant in unflooded treatments but increased under flooding (Fig. 5, 6). The increase in soluble sugars occurred more rapidly in non-mycorrhizal treatments under flooding compared to mycorrhizal seedlings (Fig. 5, 6).

Table 1. The composition of the liquid fertilizer (Hoagland's nutrient solution) used in the experiment to study the effect of root-zone flooding on mycorrhizal and non-mycorrhizal seedlings.

Mineral element	g/500 ml solution	Final concentration (µM)
$Ca(NO_3)_2.4H_2O$	118.10	5000
KNO_3	50.55	5000
$MgSO_4$	124.24	2000
KH_2PO_4	6.81	20
NaFeEDTA	1.84	100
$Na_2MoO_4.2H_2O$	0.24	0.4
H_2BO_3	3.09	20
$NiSO_4.6H_2O$	0.26	0.4
$ZnSO_4.7H_2O$	1.44	1
$MnCl_2.4H_2O$	1.98	2
$CuSO_4.5H_2O$	0.62	1
$CoCl_2.6H_2O$	0.24	0.4

Table 2. Effect of AM fungi and flooding on the mycorrhizal colonization of Passion fruit roots.

Treatments	Mycorrhizal colonization/ Days of Flooding				
	0	7	14	21	28
Mycorrhizal, unflooded	32.7±2.23	31.2±3.27	34.2±4.11	33.8±5.36	35.1±3.86
Non-mycorrhizal, unflooded	0	0	0	0	0
Mycorrhizal, flooded	34.1±4.27	32±4.37	32.5±4.37	13.7±4.37	14.6±5.28
Non-mycorrhizal, flooded	0	0	0	0	0

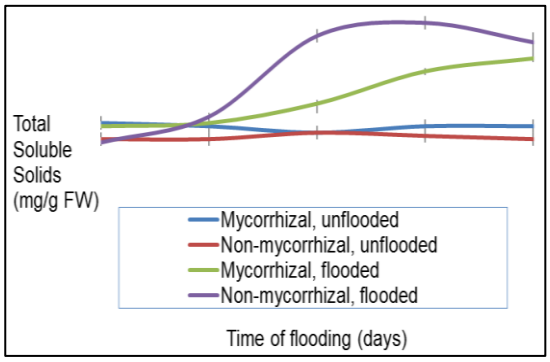

Fig. 5. Effect of AM fungi and flooding on the Total Soluble Sugars of passion fruit leaves.

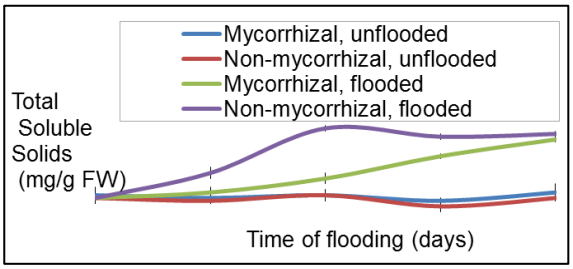

Fig. 6. Effect of AM fungi and flooding on the Total Soluble Sugars of passion fruit roots.

Leaf nitrogen content

Unflooded treatments retained constantly high leaf N content in the course of the flooding period while flooded treatments had reduced N rate starting from the 7th day in non-mycorrhizal and 14th day in mycorrhizal treatments respectively (Fig. 7). Flooded mycorrhizal treatments had significantly higher N in the 7th, 14th and 21st day of flooding compared to flooded non-mycorrhizal seedlings (Fig. 7).

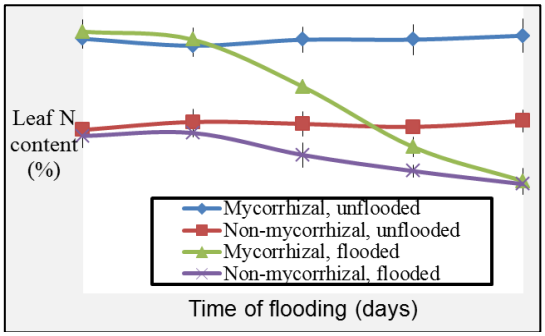

Fig. 7. Effect of AM fungi and flooding on the Leaf Nitrogen content (%) of passion fruit seedlings.

Leaf Phosphorus content

Mycorrhizal treatments had higher phosphorus content at the start of flooding (Fig. 8). The leaf phosphorus content remained constant over the next 28 days in unflooded treatments (Fig. 8). Flooding caused a reduction in the phosphorus content from the 14th day in non mycorrhizal flooded treatment.

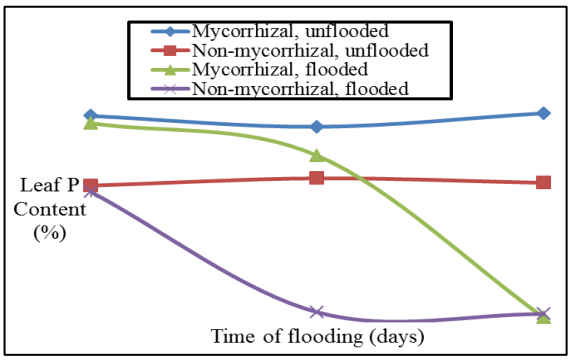

Fig. 8. Effect of AM fungi and flooding on the Leaf Phosphorus content (%) of passion fruit seedlings.

Root fresh and dry weights

The fresh and weights gradually increased in unflooded treatments but declined under flooding. Non mycorrhizal treatments had significantly lower dry weights compared to mycorrhizal treatments under flooding (Fig. 9 and 10).

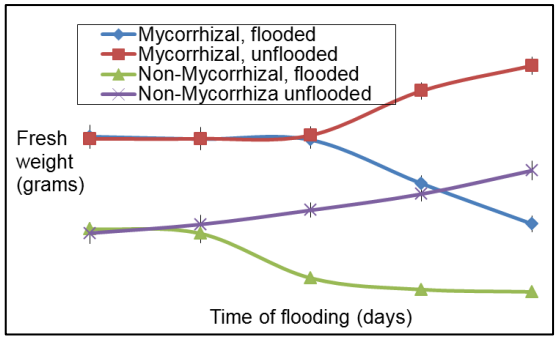

Fig. 9. Effect of AM fungi and flooding on the Root Fresh weight (grams) of passion fruit seedlings.

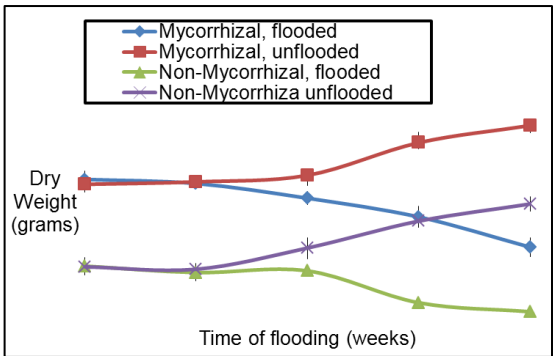

Fig. 10. Effect of AM fungi and flooding on the root dry weight (grams) of passion fruit seedlings.

Discussion

Leaf abscission occurred rapidly in non-mycorrhizal seedlings under flooded conditions. This was also reported in *Theobroma cocoa* study in Brazil which showed that the morphological effects of flooding included poor leaf expansion, limited leaf formation, leaf chlorosis, premature senescence and abscission (Carmo *et al.,* 2009).

In this study, the proline concentration increased under 14 and 21 days of flooding before falling back to the levels of unflooded treatments. This is consistent with studies which have showed that accumulation of proline in cell membrane during stress is a beneficial, acclimative response offering cellular protection against the adverse effects of stress including flooding stress (Mäkelä *et al.*, 2000). Proline also served as a hydroxyl radical scavenger (Polavarapu *et al.,* 2014) and acted as a solute that protected macromolecules against denaturation (Kishor *et al.,* 1995). In this study, flooded mycorrhizal seedlings accumulated higher proline than non-mycorrhizal seedlings.

This was also reported in *Aster tripolium* study in Portugal by Neto *et al.,* (2006) who attributed the better tolerance to flooding by AM plants to improvement of osmotic adjustment promoted by proline.

In this study, the total chlorophyll content decreased under flooding. This can be attributed to breakdown of chlorophyll under flooding. The total chlorophyll level were significantly higher in mycorrhizal treatments in the first 3 weeks of flooding indicating that mycorrhization delayed the breakdown of chlorophyll under flooding. Consistent with the reduction of chlorophyll, a reduction in the leaf nitrogen content also occurred in non-mycorrhizal seedlings under flooding. Since N is a key component of the chlorophyll molecule, the reduction of N in non mycorrhizal seedlings could have led to the reduction of chlorophyll observed in non-mycorrhizal seedlings.

As the chlorophyll content declined under flooding, the carotenoid content increased. Studies have showed that degradation of chlorophyll unmasks carotenoids, resulting in higher carotenoid expression (Gross, 1991). Mycorrhizal inoculation delayed the increase in carotenoid content possibly maintaining higher nitrogen content in the leaves, which in turn ensured greater chlorophyll expression.

Mycorrhizal root colonization remained constant under unflooded conditions but under flooding, almost 50% decline in colonization was observed, starting 21 days after flooding. Soya beans study in Japan also showed that AM colonization ratio reduced from 12.5% (in the primary and lateral roots) and 14.5% (in the adventitious roots) in unflooded treatments to 0.8% and 7.5% in flooded treatments respectively (Hattori *et al.,* 2013).

The reason for this reduction in colonisation under flooding is because arbuscular mycorrhiza fungi are obligate aerobes and colonization was suppressed when soil moisture became too high or low (Entry *et al.,* 2002). This is because (Reid and Reid, 2008). However, in this study, the low colonization under flooding still conferred significant benefit to the passion fruit seedlings. In this study, the total soluble sugars increased sharply under flooding.

These findings agree with studies in maize carried out in Iran which indicated that the amount of soluble sugars increased 1.5-2 times when compared with unflooded controls during early stage of flooding (Pourabdal *et al.,* 2008). The increase in total sugar content was more rapid in non mycorrhizal treatments and this can be attributed to increased sugar accumulation in the leaves due to reduced translocation to the roots (Barta, 1987). It can also be as a result of reduced carbohydrate utilization in roots (Wample and Davis, 1983) or to depression of the photosynthate transport system (Topa and Cheeseman, 1992).

The increase in soluble sugar content by mycorrhizal plants subjected to flooding was also reported by Neto *et al.,* (2006). In a study of *Aster tripolium* in Portugal Neto *et al.,* (2006) showed that mycorrhizal plants had better tolerance to flooding that was mediated through improvement of the osmotic adjustment of the plant tissues via production of higher concentrations of soluble sugars.

In this study, the leaf nitrogen content remained constant under unflooded conditions. Flooding however caused a reduction in the leaf nitrogen content with non-mycorrhizal seedlings showing more rapid decline. The total nitrogen content in plant tissue has been reported to decrease under flooding stress in various crop species, including apples (Hsu *et al*., 1999). Flooding also caused a reduction in the phosphorus content with mycorrhizal plants maintaining higher P content. The higher N and P content may be related to the greater root fresh and dry weights observed in mycorrhizal seedlings under flooding. This translated into better root health promoted by mycorrhization therefore facilitating uptake of nutrients, and ensuring higher nutrient content in the leaves.

This study found out that increased production of proline, maintenance of optimum nutrient supply in the leaves and maintenance of leaf chlorophyll aid mycorrhizal passion fruit seedlings to delay the adverse effects of flooding.

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