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Application of Amino Acids on Philippine Native Tomato (*Lycopersicon esculentum* L.) Grown Under Flood Condition

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ABSTRACT

Flooding is the most important abiotic stress in the tropical and subtropical regions that negatively affects terrestrial plant growth and productivity and even leading to plant death. Amino acid accumulation, on the other hand, may serve as defense during stress resulting to tolerance among others. Thus, a study was conducted to determine the growth, root length and number of leaves produced by Philippine native tomato as affected by different levels of amino acid under varying durations of flooding. Data were statistically analyzed through Analysis of Variance (ANOVA) for Randomized Complete Block Design (RCBD). Comparisons among means were done using Duncan's Multiple Range Test (DMRT). Shoot growth was increased with application of amino acids (100 ppm) after both flooding conditions (three and six days) at 3.28cm and 7.47cm, respectively. Moreover, no amino acid application or minimal amino acid application (100 ppm) produced the most number of leaves on unflooded and minimally flooded conditions (three days only). On root length, longer roots were produced by plants without amino acid (12.68 cm) or lesser amino acid (100 ppm) at 9.43 cm, while flooding had no significant effect on root length. These results clearly indicate the value of exogenous amino acid (100 ppm) application mainly on shoot growth of tomato. Furthermore, exogenous amino acid application is deemed necessary because even in both number of leaves and root length where results are comparable to without amino acid application, no negative effect was observed.

Keywords **-** agriculture, flooding, amino acid, Philippine native tomato

INTRODUCTION

Flooding, which includes soil waterlogging and submergence is one of the most frequent and extensive abiotic stresses that negatively affect terrestrial plant. In tropical and subtropical regions, severe crop losses are caused by prolonged seasonal rainfall. Excess water produces anoxic soil conditions within a few hours (Gambrell and Patrick, 1978). Climate change is likely to cause an increase in the occurrence of heavy rains leading to flooding of agricultural lands (Vidoz et al., 2010). The main cause of damage under waterlogging is oxygen deprivation, which affect nutrient and water uptake, so the plants show wilting even when surrounded by excess of water (Sairam et al., 2008). The 10^4-fold decrease in the gas diffusion rate in water compared to air negatively affects oxygen supply, and causes accumulation of ethylene in submerged tissues. It is ethylene production and entrapment that trigger a number of plant responses to flooding and submergence. These responses vary with plant species, and include epinasty, hyponastic growth, shoot elongation, aerenchyma formation and adventitious root development (Vidoz et al., 2010).

Tomato (Lycopersiconesculentum L.) is one of the most important vegetable crops in the world. It is the second most important vegetable crop, from an economic perspective, after potato, and the fresh tomato trade has increased by 45% in the last five years. However, it is highly flood-sensitive (Vidoz et al., 2010). Philippine native tomato variety is believed to tolerate and adapt mild abiotic stresses such as flooding and drought as it has been suggested that physiological and molecular studies of the mechanisms of anoxia tolerance in wild or native plants that still possess long-term anoxia-tolerance are more likely to provide evidence of physiological mechanisms than studies of crop plants (Crawford and Braendle, 1996).

Furthermore, applications of amino acids are also known to improve tolerance of particular crops to some abiotic stresses. Amino acids such as threonine serve as defense mechanism during stress; serine increases stress tolerance and forms humic compounds; valine increases drought tolerance and seed establishment; isoleucine increases tolerance to saline stress; leucine increases saline tolerance and pollen germination; and histidine regulates root development and stress tolerance (Agrihortiplantbooster, as cited by Agustin, 2009). These amino acids are readily available through commercial amino acid tablet in the market.

Thus, a study that uses amino acid on native tomatoes under flooded condition is deemed necessary in understanding both the extent of tolerance of native tomato to flooding and the effects of exogenous amino acid application on flood tolerance of tomato.

MATERIALS AND METHODS

Sowing of Seeds

Philippine Native Tomato variety was used. All healthy and vigorous seeds of tomatoes were sown in a sowing box. It has an average height of 37- 83 cm at flowering, containing branches ranging from two to seven upon flowering and is viny (creeping). It normally flowers 30- 53 days after transplanting and yields up to 0.23- 1.3 tons/ha.

The Philippine Native Tomato used in the study is shown in Figure 1 and additional images are shown as Plates.

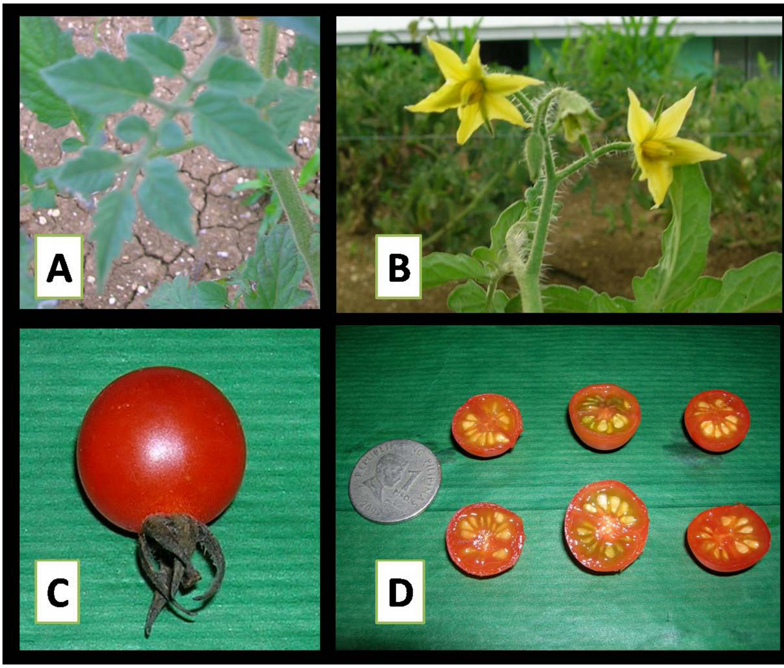


Figure 1. Philippine native tomato with its recognizable parts: (A) leaves (B) flowers (C) fruit and (D) cross-section fruits

Transplanting of Seedlings

Transplanting of native tomato seedlings was done one week after sowing, placing two healthy plants per bottle by using forcep.

Preparation of Amino Acid

Commercial amino acid tablet (Superior Amino Acid 2222) was used. The tablets were crushed into powder using mortar and pestle.

Each tablet has the following amino acids with corresponding amount:

Table 1. Amount of amino acids per one Superior Amino Acid 2222 tablet

Amino Acids	Amount per tablet (mg)	Amino Acids	Amount per tablet (mg)
Tryptophan	40	Cystine	30
Valine	130	Tyrosine	70
Threonine	109	Histidine	40
Isoleucine	130	Proline	130
Leucine	240	Glutamine	388
Lysine	159	Aspartic acid	239
Phenylalanine	89	Serine	100
Methionine	40	Glycine	69
Arginine	120	Alanine	99

The treatments were achieved by dissolving 100 mg of Superior Amino Acid 2222 powder in one liter water (100 ppm) and dissolving 200 mg of the same tablet in one liter water (200 ppm) applied only once, one week after transplanting or one week prior to imposition of flooding.

Preparation of Growth Medium

Twenty seven clean transparent bottles with 500 ml capacity were used. Each bottle was filled with 50ml of composite vermi-compost medium produced by Cebu Technological University – Barili Campus.

Experimental Design and Field Layout

A 3x3 factorial experiment arranged in Randomized Complete Block Design (RCBD) with three replications was used in the study. Treatments were formulated by the researchers to probe the level of amino acids that will elicit favorable response of tomatoes. No previous study was used to come-up with the range of amino acids used.

The treatments were as follows:

Factor A – Amino Acid

A_0 – Control (No amino acid)
A_1 – 100 ppm amino acid immediately after flooding
A_2 – 200 ppm amino acid immediately after flooding

Factor B – Flooding

F_0 – Control (No flooding)
F_1 – Flooding for three days up to 5 cm from the base of the plant two weeks from transplanting
F_2 – Flooding for six days up to 5 cm from the base of the plant two weeks from transplanting

Irrigation

After flooding, soil moisture content was maintained at normal using 4 in 1 Soil Survey Instrument KC300 pH Meter.

Data Gathered

Shoot Growth

Shoot growth (cm) was measured as the difference between the existing plant height and the initial plant height.

Root Length

Root length (cm) was measured from the origin of top most root emergence up to the tip of the longest root. This was done during the termination of the study.

Number of Leaves

Number of leaves was done by counting the number of true opened leaves during the first, second (termination) week after exposure to flooding.

Statistical Analysis

Data were recorded, tabulated, consolidated and statistically analyzed through Analysis of Variance (ANOVA) for Randomized Complete Block Design (RCBD). Comparisons among means were done using Duncan's Multiple Range Test (DMRT) to determine the specific significant differences among treatments.

RESULTS AND DISCUSSION

Plants treated with 100 ppm amino acid showed promising result in flooded condition (both three and six days) as it produced the highest shoot growth among all treatments and its number of leaves and root length was comparable to plants with no amino acid treatments.On the other hand, plants exposed to flooding for about six days and treated with amino acid at 200 ppm exhibited some mortality.

Shoot Growth (one week after flooding)

Table 2 shows that for individual effect, only amino acid level significantly influenced shoot growth, the highest of which is with the application of 100 ppm amino acid. Furthermore, significant interaction was also noted on the results, it revealed that the highest growth was observed on flooded plants (3 days) and applied with 100 ppm amino acids. Pascual*et al.* (2013) reported that leaf length of pechay was increased 1.77 times when applied with fermented activators and EM solution. Both of which are known to contain substantial amount of amino acid. Furthermore, this may attributed to proline accumulation wherein plants subjected to stress show accumulation of proline and other amino acids (Rai, 2002) which contributes to osmotic adjustments and tolerance to plants exposed to unfavorable environmental conditions (Claussen, 2004). However, excessive amount (200 ppm) may be more harmful than beneficial to plants.

Table 2. Shoot growth (cm) as affected by different level of amino acids and duration of flooding one week after flooding

Amino Acids (ppm)	Flooding (number of days)			Mean
	0	3	6	
0	2.37[b]	2.10[b]	2.47[b]	2.31[b]
100	2.25[b]	4.12[a]	3.47[ab]	3.28[a]
200	2.17[b]	2.65[ab]	0.00[c]	1.61[b]
Mean	2.26	2.96	1.98	2.40

Means not sharing letter in common differ significantly by Duncan's Multiple Range Test (DMRT) at 5% level.

Number of Leaves (one week after flooding)

A decrease in the number of leaves was observed upon application of increased amino acid (200 ppm) or prolonged flooding (six days). This indicates that flooding to three days will not significantly affect the capacity of the native tomato plants to develop leaves and amino acid application (100 ppm) does not improve nor negatively affect that plant development capacity as shown in Figure 2.

Shoot Growth (upon termination)

Upon termination (Table 3 and Plate 1), for individual effect, both amino acid level and flooding duration significantly influenced shoot growth, the highest of which is with the application of 100 ppm amino acid and the least are those subjected to flooding for six days. Furthermore, significant interaction was also noted, it revealed that highest shoot growth was observed on flooded plants (six days) applied with 100 ppm amino acids. This implies the value of amino acid application on plants exposed to flood conditions. Rai (2002) reported that amino acid such as proline accumulation aids in K^+ accumulation in *Vigna radiate* cultures.

Means not sharing letter in common differ significantly by Duncan's Multiple Range Test (DMRT) at 5% level.

Figure 2. Number of leaves as affected by different level of amino acids (A) and duration of flooding (B) one week after flooding

Legend:

Factor A
A_0 – Control (No amino acid)
A_1 – 100 ppm amino acid immediately after flooding
A_2 – 200 ppm amino acid immediately after flooding

Factor B
F_0 – Control (no flooding)
F_1 – Flooding for three days up to 5 cm from the base of the plant two weeks from transplanting
F_2 - Flooding for six days up to 5 cm from the base of the plant two weeks from transplanting

Table 3. Shoot growth (cm) as affected by different level of amino acids and duration of flooding upon termination of the study

Amino Acids (ppm)	Flooding (number of days)			Mean
	0	3	6	
0	5.52^{ab}	4.22^{b}	5.63^{ab}	5.12^{b}
100	7.93^{a}	6.80^{ab}	7.67^{a}	7.47^{a}
200	7.95^{a}	6.90^{ab}	0.00^{c}	4.95^{b}
Mean	7.13^{a}	5.97^{ab}	4.43^{b}	5.85

Means not sharing letter in common differ significantly by Duncan's Multiple Range Test (DMRT) at 5% level.

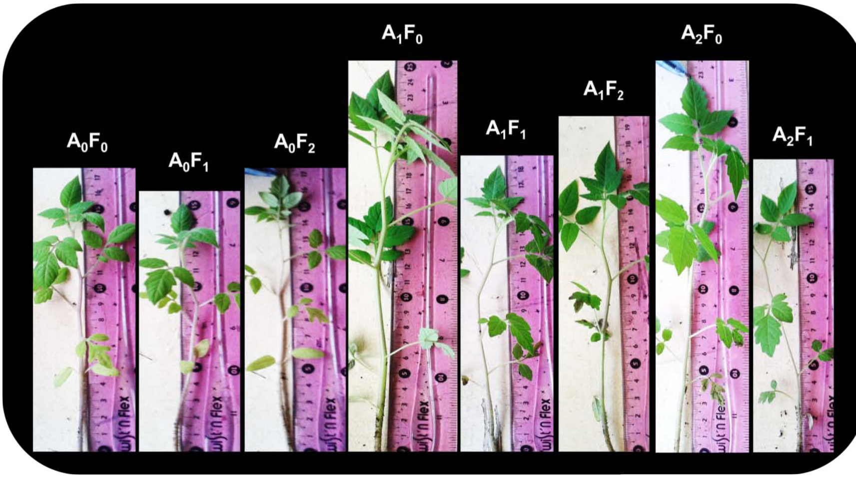


Figure 3. Shoot growth as affected by different level of amino acids and duration of flooding upon termination

Root Length (during termination)

With respect to root length during termination (Table 4 and Plate 2), longest roots was on plants without amino acid application under flooded condition (14.85cm), while the shortest were recorded on those applied with 200ppm and flooded for three days (7.27cm). This gives us an idea that native tomatoes shows flood resistance since flood susceptible plants do not change its vertical root distribution (Sairam, et al., 2008). Moreover, this also gives a good insight on the influence of amino acid application on flood tolerance since it yielded to shorter root system. This maybe because amino acid increased the adaptive mechanism of the plant as new roots replace the function of the original root system (Jackson and Drew, 1984) thus no further increase in root length were observed. Under flooded conditions, plant roots are in a state of hypoxia, their metabolic activity is inhibited and ATP production decreased (Saglio et al., 1980). The decreased ATP production restricts the supply of energy for root growth, thus reducing vegetative growth (Liao and Lin, 2001).

Table 4. Root length (cm) as affected by different level of amino acids and duration of flooding upon termination of the study

Amino Acids (ppm)	Flooding (number of days)			Mean
	0	3	6	
0	11.35^{ab}	11.83^{ab}	14.85^{a}	12.68^{a}
100	7.30^{b}	10.00^{ab}	11.00^{ab}	9.43^{ab}
200	11.67^{ab}	7.27^{b}	0.00^{c}	6.31^{b}
Mean	10.11	9.70	8.62	9.47

Means not sharing letter in common differ significantly by Duncan's Multiple Range Test (DMRT) at 5% level.

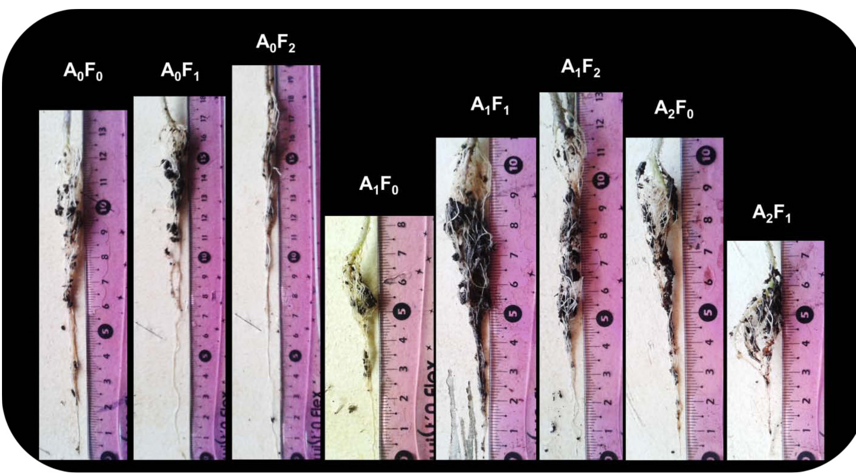
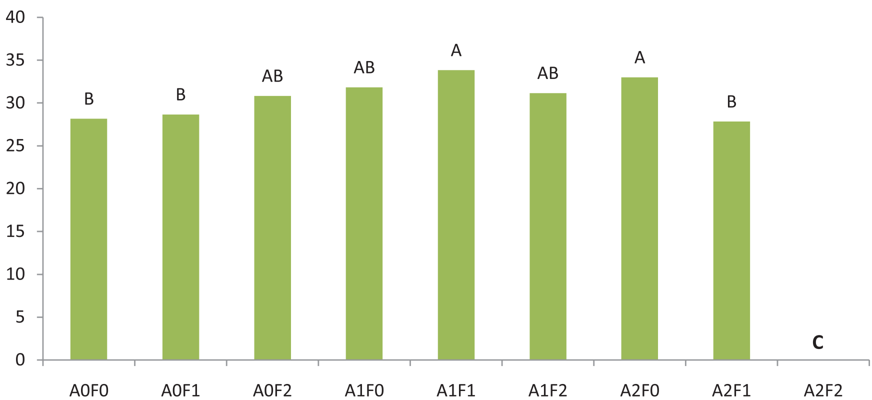


Figure 4. Root length as affected by different level of amino acids and duration of flooding upon termination

Number of Leaves (upon termination)

There is a decrease in the number of leaves under varying flooding levels as presented in Figure 3. Plants under three days of flooding two weeks from transplanting were comparable to plants without flood stress as highest number of leaves appeared but plants under six days of flooding got the lowest number of leaves. This may be due to the negative influence of flooding to the plant (Vidoz et al., 2010). On the other hand, plants treated with 100 ppm of amino acid got the highest number of leaves but were comparable to plants without amino acid application which is higher compared to plants with 200 ppm of amino acid. This is supported on the study on pechay (*Brassica pekinensis* L.) in which number of leaves were increased by 0.78 times as applied by commercial EM or fermented activators containing amino acids (Pascual et. al., 2013).



Means not sharing letter in common differ significantly by Duncan's Multiple Range Test (DMRT) at 5% level.

Figure 3. Number of leaves as affected by different level of amino acids and duration of flooding upon termination

Legend:

Factor A	Factor B
A_0 – Control (No amino acid)	F_0 – Control (no flooding)
A_1 – 100 ppm amino acid immediately after flooding	F_1 – Flooding for three days up to 5 cm from the base of the plant two weeks from transplanting
A_2 – 200 ppm amino acid immediately after flooding	F_2 - Flooding for six days up to 5 cm from the base of the plant two weeks from transplanting

CONCLUSIONS

Flooding affects the growth, root length and number of leaves formation of Philippine Native Tomato at three and six days. On the other hand, application of amino acid (100 ppm) provides promising beneficial effects especially on shoot growth without negatively affecting root growth and development of leaves. Further studies should be conducted considering lower amounts of amino acids. It is also recommended that long term effect of incorporation of amino acids and extending observation period till fruiting and harvesting stage to examine effect of amino acids to fruits be studied.

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