Arginase in Seeds and Seedlings of Five Varieties of Tomatoes (*Solanumlycopersicum* L.)

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Abstract: **- This research was carried out to evaluate the quantities of arginase present in the seed and seedlings of Tomato plant (*Solanumlycopersicum*).The Tomato fruits used in this study were collected from Oye market, Oye town in Ekiti State, Nigeria. The fruits were identified in the Herbarium section of Plant Science and Biotechnology laboratory, Federal University Oye-Ekiti. Samples of *S.lycopersicum* were homogenized, buffered using citrate buffer and centrifuged, arginase assay protocol was conducted and its concentration was determined at wavelength of 450nm. The Roma variety was discovered to have highest absorbance of arginase, highest specific activity and the highest yield percentage value in comparison to the Cherry, Amish paste, Early girl and Pear varieties. The research may have established that the Roma variety of *S.lycopersicum* give better yield and have a good defensive mechanism for pathogenic attacks due to its high arginase concentration.**

Keywords: ***Solanumlycopersicum*****, Arginase, Specific Activity, seeds (sd), seedlings(sdl).**

I. INTRODUCTION

Tomato is one of the most important vegetable crops grown all over Nigeria and in the tropical and subtropical belts of the world. It is the world's largest vegetable crop after potato and sweet potato but it tops the list of canned vegetables. It is an important condiment in most diet and a very cheap source of vitamins, calcium and niacin all of which are of great importance in the metabolic activities of man. Tomato is a good source of vitamins A, C and E and minerals that are very good for body and protect the body against diseases (Olaniyi *et al.,* 2010).

Tomatoes have also been found to be important for human life in many ways. Tomato is ranked at the top of all fruits and vegetables as a source of vitamins and minerals in the world (Eurostat statistics, 2016). Tomatoes are widely known for their outstanding antioxidant content; they also have anti-cancer potential which comes from being a non-starchy vegetable as well as a source of vitamin C and carotenoids (American institute for Cancer Research, 2017).

Tomatoes contain proteinaceous compounds and these include enzymes such asarginase,β-1,3-glucanases and chitinases, a-amylases and also other proteins and peptides which are responsible for their antimicrobial activity and have helped them to develop protective mechanisms that enable them to successfully resist different unfavorable conditions (*Ines and Marcos, 2013*; *Candido et al., 2011*).

Arginase is one of the key enzymes in urea cycle, and it is proved to be important in ammonia detoxification (*Flores et al., 2008*). The reaction products may be further metabolized to other amino acids and polyamines, including proline, putrescine, spermidine, and spermine, all of which play crucial roles in wide developmental processes and in biotic and abiotic stress responses (Wang *et al.,* 2011b; Brauc *et al.,* 2012; Shi*et al.,* 2014).

Some of the best sources for enzymes are fresh fruits, vegetables, and sprouted grains. Just as the body needs enzymes to function, so do plants need enzymes for growth, reproduction, and health. Foods are such rich sources of enzymes that some enzyme supplements are actually derived from food sources. All fresh fruits, vegetables, or grains are potential enzyme sources, but only if the enzymes have not been destroyed by heat, radiation, or any of the other processes. Arginase has also been reported in some plants like tomatoes, apple, water melon, oranges etc.in varying distribution (Okonji R. E. and AgboolaO. S. 2014).

This study therefore aimed at making available information on the comparative assay of the presence of an important metabolic enzyme precisely arginase in five tomato varieties and to ascribe possible roles for the enzymes in the varieties they are present.

The varieties of *Solanumlycopersicum* used for the research were *Roma/plum* tomatoes, *Cherry* tomatoes, *Pear* tomatoes, *Amishpaste* tomatoes, *Earlygirl* tomato. The Roma variety is also known as Plum tomato. These are small and egg-shaped; they tend to be less juicy than slicing tomatoes. They are thick and contain fewer seeds than the other tomato variants used. They are locally called "*tiwantiwa*". Cherry tomato variety are rounded and mostly red although other variety colours exist. These tomatoes tend to be just about same size as a cherry and are very juicy. It is locally called "*Alausa*". Also, Pear variety got their name from their shape which resembles a pear. They do not have much juice. There are yellow, orange and red varieties of this tomato. It is locally called "*Gboro*". The Amish paste variety is a tomato of Amish origin. It is also juicy. It is locally referred to as

"*Olomi*". Early girl tomatoes are medium-sized globe type. They have bright colours and quite firm. They are less juicy. They have lines of symmetry around their fruits. It is locally called "tomato igbo".

Statement of Problem

Many plant growers encounter challenges in breeding plants such as tomatoes as there might be poor uptake of nutrient, susceptibility pests and pathogens, poor yield in the field etc. Research has shown that arginine and its products remediates these problems. Therefore, it becomes important to know the tomato variety that has increased level of arginine.

Plant enzymes are protein molecules in cells of plants that work as catalysts and catalyse chemical and physiological reactions necessary for growth, development, repair and maintenance of the plant but do not get used up in the process (Pekka Mäntsälä and Jarmo Niemi, 2009). Generally, an enzyme is a biological catalyst that speeds up the rate and efficiency of chemical reactions in living organisms.

The enzyme acts upon substrates and convert them into various molecules referred to as products. An enzyme is a protein but not all proteins are enzymes (Maximum Yield, 2017). Plant enzymes enhance plant growth by creating additional energy for the plant to grow big and strong even under harsh conditions, it creates a stronger root system. Plant enzymes are numerous such as rhodanase, arginase, thiaminase, 3-Mercaptopyruvate Sulphurtransferase etc. (Ehigie*et al.,* 2013). These enzymes perform vital roles in the physiological growth and development of plants.

Arginase

Arginase (also known as arginine amidinase, canavanase, L-arginase, arginine transamidinase) is a crystalline semi-essential manganese-containing enzyme that converts naturally occurring arginine into ornithine and urea. (*www.merriam webster.com*). Arginase is one of the important enzymes in urea cycle and is important in ammonia detoxification (Mohamed SA *et al*., 2005; Mavri-Damelin *et al., 2007*: Peters SJ *et al*., 2008).

The reaction products may be further metabolized to other amino acids and polyamines, including proline, putrescine, spermidine, and spermine, all of which play crucial roles in wide developmental processes and in biotic and abiotic stress responses (Flores *et al.,* 2008; Brauc *et al.,* 2012; Shi*et al., 2014*).

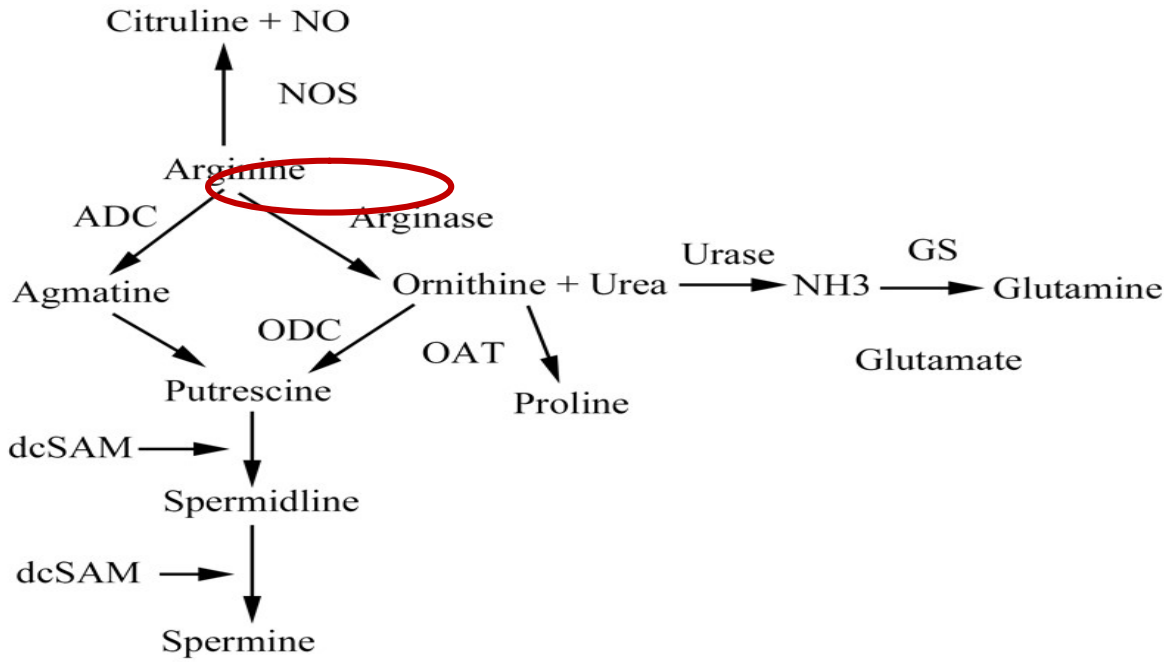

Figure 1 The pathway of Arginine Metabolism in plant

Source: www.wiki.com

L-ornithine is the precursor of polyamine synthesis; urea, a precursor of nitrite, nitrate and NO synthesis is involved in nitrogen metabolic cycle (Brownfield *et al.,* 2008). Polyamine produced as a result of breakdown of ornithine produced from arginase activities serves as a scavenger of reactive oxygen species (ROS) and a stess signal in plants regulating plant growth as well as biotic and abiotic stress (Wang *et al.,* 2011; Wimalasekera *et al.,* 2011).

It has been proved that majority of environmental stress such as dehydration, high salinity, chilling and other stresses will result in excessive accumulation of ROS quickly in plants which leads to oxidative stress and may lead to death (Mittler2006; Miller *et al*., 2010; Zhu *et al.,* 2002, Zhu *et al*., 2016). Plant development and a range of biotic and abiotic stress responses can be regulated by NO and polyamines in plants (Ji-Hong Liu *et al*., 2015; Lozano-Juste *et al.,* 2011; Wang *et al.,* 2011).

In plant tissues, arginase plays various physiological and metabolic role including nitrogen mobilization during fruit development, seed germination, biosynthesis of glutamine and polyamines, degradation of urea and participation in plant responses to herbivorous insects since it catalyses transformation of essential protein amino acid, arginine to non-protein ornithine (Cezary *et al.*2008).

Arginine is one of the most functionally diverse amino acids in living cells. In addition to serving as a constituent of proteins, arginine is a precursor for biosynthesis of polyamines (PAs), Agmatine and proline as well as the cell signaling molecules glutamine and nitric oxide (NO). In higher plant, it has also been proposed that both endogenous and exogenous arginine have roles in plant stress response, such as salinity, water and disease (Nasibi*et al*., 2011; Zheng*et al.,* 2011).

A research showed the role of arginine in alleviating the effect of salinity stress on mung beans (*Vigna radiate* L.Wilczek).Salinity stress reduced most yield components and nutritional value of produced seeds. However, spraying mung bean plants with arginine alleviated the harmful effect of salinity on plant height and plant dry weight. (Abd El-Monem, 2007).

Exogenous application of polyamine (a product of arginine), formed by indirect effect of arginase, to several plantspecies have been shown to promote cell division, celldifferentiation and growth. (Xu*et al*. 2001; Nassar*et al*., 2003). Study showed that putrescine, a product of arginine was exogenously suppliedon the salt stressed plant, the grain yield of wheat increased (El-Bassiouny and Bekheta2001)

In a research work, the application of arginine significantly promoted the growth and increased the fresh and dry weights, certain endogenous plant growth regulators, chlorophylls a and b and carotenoids in bean (*Vignasp*) (Nassar *et al.,* 2003); in wheat (AbdElMonem *et al.,* 2007; El-Bassiouny*et al., 2*008). It was observed induced disease resistance in tomato fruits via its effects on nitric oxide biosynthesis and defensive enzyme activity (Zheng*et al*., 2011).

The alkalinization of the cytosol by induction of arginase and urease activity due to rapid hydrolysis is an active component of pathogen defense mechanisms (Polacco *et al.,* 2013). Overproduction of arginase in plants provide enhanced resistance to herbivores by acting as an anti-nutritive defense against phytophagous insect (Gregg A. Howe and Hui Chen,2011). It has been proved that arginase along with threonine deaminase (TD) induced by Jasmonic acid inducible protein (JIP) in *S.lycopersicum* plants attacks arginine in the mid-gut causing intestinal issues in *Maducasexta* larvae which infest the tomato plant hence conferring plant protection against herbivores (Chen*et al.,* 2004).

Treatment of *S. lycopersicum* fruit with hot air at 38°C enhanced the transcript levels of LeARG1 and LeARG2, the two genes encoding arginase, and thus arginase activity. Results showed that arginase induction may be partly involved in HA-induced chilling tolerance in tomato fruit, possibly by a mechanism involving activation of antioxidant enzymes and an increase in proline levels (Chen *et al.,* 2004).Treatment of *Solanumlycopersicum* with methyl jasmonate enhanced transcription levels of arginase genes. Results showed that arginase is involved in methyl jasmonate -induced chilling tolerance of tomato fruit, possibly by ameliorating the antioxidant enzyme system of fruit and increasing proline levels (Chen *et al.,* 2004). The physiological role of arginase in physiologically and metabolically has been well described in several species of plants.

II. MATERIALS AND METHODS

Fruits Collection and Seeds Extraction

The fruits of *Solanumlycopersicum* were purchased from Oye market, Oye town in Ekiti State. They were collected in two batches enough for the study and identified using the tomato identification protocol provided. The fruits used for seed extraction were those at full maturity. The seed viability depends on the method with which seeds are extracted, therefore, it is important to choose proper methods of seed extraction.

The ripe tomato fruits were collected, crushed in plastic buckets and kept 2 days to ferment at room temperature to make viable seeds settle down at the bottom. The mixture was then stirred and the flesh pulps were discarded into disposable nylons and clean water was poured into the bowl containing the fruit content to separate the seeds from the other contents of the tomatoes. The mixture was stirred continuously and left to settle for a while, after which the water above was poured out and clean water was poured into the seeds below to obtain clean seeds. Properly washed seeds were arranged in open labelled petri dishes on a laboratory shelf at room temperature and left for 3days to dry properly.

Germination Studies

Pre-germination test was then carried out on the extracted seeds of five varieties of *Solanumlycopersicum*. Five germination plates were properly cleaned and arranged on a lab bench. Tissue paper in place of filter paper was used as substratum, 3ml of distilled water was added to each plate and covered. After which they were transferred to the laboratory

shelves for further growth under laboratory conditions. Emergence of radicles and plumules were recorded as evidence of germination..

Protein and Enzyme Assays

After the varieties' seeds were soaked in 0.1M citrate buffer and kept in the fridge for a while to weaken the cells. Seeds were then homogenized using chilled mortar and pestle (enzymes surface in cold environment) and poured into bottles with lid. Bottles were kept in fridge overnight (to allow buffer to move in and out) and centrifuged at 4,000rpm for 20mins. Supernatants were collected and stored in cold box and pellets were discarded.

Protein Assays:

The protein determination was carried out using Bradford method (1976).All chemical analysis were performed in two replicates for each variety. 100µl of samples were pipetted into well labeled test tubes with 1000µl of Bradford reagent. Protein content for each variety were then checked under UV Spectrophotometer at 595nm.

Arginase assays:

Arginase activity was determined according to the method of Kaysen and Strecker (1973). All chemical analysis were performed in two replicates for each variety. 200µl of carbonate buffer, 50µl of 0.001M $MnCl_2$of pH 9.5, 150µl of Arginine and 100µl of sample were pipetted into well labelled test tubes and incubated for 10mins at room temperature. Then, 250µl of Ehrlich reagent was added to each test tube and absorbance was analysed under UV spectrophotometer at 450nm. The unit of activity of arginase is defined as the amount of enzyme that will produce one µmol of urea per min at 37^{o}C.

Precautions taken during the Protein and Arginase Analysis of Solanumlycopersicum

Samples were stored in the freezer before the extraction process. Citrate buffer was added to homogenized samples to resist changes in pH of protein solution, else, there might be denaturing or precipitation of protein. Apparatus like mortar and pestle were refrigerated to allow reduced denaturing of extracts. Crude extracts were kept under refrigeration to stop proteolytic activity of proteases. Centrifuged samples were kept in a cold box to prevent denaturing by heat. Stopwatch was used in ensuring accurate timing. The cuvette of the UV spectrophotometer was rinsed in distilled water and cleaned with tissue paper before each sample was poured and analyzed.

Evaluation of Some Physiological Parameters

Visual observations and data collections were carried out as regards the emergence of radicle and plumules from seeds. Also, the root length of the five varieties of *Solanumlycopersicum* varied from one variety to another.

III. RESULTS

Table 4 shows the five varieties of *Solanumlycopersicum* assessed and their different protein concentrations. Amish paste was observed to have the highest protein absorbance, hence, the highest concentration. Also, the table 1 shows the absorbance and specific activity of arginase in the seeds and seedlings of the five tomato varieties. Arginase activity and absorbance was highest in both seeds and seedlings of the Roma variety of *S. lycopersicum*.

The Protein Concentration

Figure 2 shows the comparison of protein content among the five varieties of *S.lycopersicum*. Amish paste was observed to have the highest protein absorbance in the seeds when compared to theother four varieties of *S.lycopersicum*.

Arginase Activities

Figures 3 & 4 show the comparison of arginase activities in seeds and seedlings among the five varieties of *Solanumlycopersicum*. It was observed in this study that arginase activity and absorbance is highest in both seeds and seedlingsof Roma variety of *S.lycopersicum*.

Growth of Seedlings and Root Development

The growth of the seedlings of the five varieties of *Solanumlycopersicum* was evaluated, Roma variety was observed to have the highest percentage yield of 73% in comparison to the other four varieties: Cherry variety (54.5%), Amish-paste variety (48.6%), Early girl variety (40.5%), Pear variety (32.4%). Also, average performance of the five varieties of *S.lycopersicum* showed that Roma variety had the longest tap root system.

$$\% \text{ of seedlings' growth} = \frac{\text{No of germinated seeds}}{\text{Total no of seeds sown}} \times \frac{100}{1}$$

IV. DISCUSSION

Five varieties of *Solanumlycopersicum* were used in this study and arginase concentration was compared as in Table 2 above. Nitrogen has been reported to limit plant growth, and arginine has the highest nitrogen to carbon ratio among 21 amino acids and plays a key role in the growth of plants (Gudrun *et al.,* 2015). The variety with the highest arginine level will in turn have the highest arginase content. This corresponds with the Roma variety having the highest percentage yield (73.0%) and Pear variety having the lowest percentage yield (32.4%) in Table 3 above due to its its lowest arginase content in seedlings**.**

High content of arginine in plants regulates plant susceptibility to pest and pathogen attack (Cezary*et al*., 2008). So, from the result obtained, it can be deduced that the Roma variety has a higher pathogen defense system. This corresponds with the thick pulp of the fruits of this

variety.Arginase participate in plants responses to herbivorous insects since it catalyses transformation essential protein amino acid – arginine to non-protein ornithine (Cezary*et al*., 2008)**.**

The higher the Arginase content, the stronger the resistance of the variety against pathogen attack and stress in relation to Gregg Howe and HuiChen, 2011 research. It has been shown in many studies that arGrow (arginine fertilizer) treated plants or plants with high arginine level form better improved root systems with optimal nitrogen level which results in faster establishment and growth of plants and higher average survival rate (SweTree Technologies, 2014). This aligns with the fact that the Roma variety has the highest germination percentage in Table 3 above and also, had highest average performance of root system.

Most studies on plant arginase have focused on its role in mobilizing arginine during early seedling germination. Storage proteins are mobilized to provide amino acids for protein synthesis in the plant. Urea and ornithine formed in higher plants as products of arginasereaction are used in the assimilation of nitrogen into amino acids andbiosynthesis of polyamines that enhance plant development and root system. (Okonji*et al.,* 2014.)

Thus, Roma variety of *Solanumlycopersicum* could be considered for further improvement. Since it has also been discovered that arginine gives rise to sturdy plants and fruits and this explains the fact that Roma variety has the highest arginase content and the thickest fruit flesh (*SweTreeTechnologies, 2014*).

Arginase was discovered to be involved in methyl jasmonate -induced chilling tolerance of tomato fruit, possibly by ameliorating the antioxidant enzyme system of fruit and increasing proline levels and thus attacking homeostasis and chilling stress. Thus, the higher the concentration of arginase the higher its stress tolerance. According to this study, Roma has the highest concentration of arginase with the highest specific activity ($14.38 \times 10^{-3}$and 5.4×10^{-3} in seeds and seedlings respectively) and absorbance (0.623 and 0.234 in seeds and seedlings respectively) in relation to the other four varieties as in Table 2 above.

In a study, it was observed that the Roma variety in addition to arginase content has the highest amount of lycopene in comparison to three other varieties (Cynthia H. and Farah F., 2006), and this lycopene has been said of tomatoes to cure prostate cancer and reduce cholesterol (Ravi Teja, 2017; Jessie Szalay 2016). This further supports that the Roma variety is of a good advantage in plant breeding.

Exogenous arginine can also be added to *Solanumlycopersicum* to further enhance arginase activities and in turn promote field yield, pathogen defense system, root system, sturdiness, nitrogen storage, fruit ripening increased growth in the field.A research showed the role of arginine in alleviating the effect of salinity stress on mung beans (*Vigna radiate* L.Wilczek).Most yield components and nutritional value of produced seeds reduced due to salinity stress. However, spraying mung bean plants with arginine alleviated the harmful effect of salinity on plant height and plant dry weight. (Abd El-Monem, 2007).

The variability observed among the above named five varieties of tomatoes shows that there are potential for improvement in tomatoes.

It should be noted that high concentration of arginine result in high absorbance for arginase.

V. CONCLUSION AND RECOMMENDATION

On the basis of the variability of these five varieties assessed, it can be deduced that there is visibly a wide range of selection for tomato breeders. It can further be established from the study that it could be beneficial to select healthy seeds, preferentially from the *Roma* variety of *S. lycopersicum* for breeding and hybridization other than the other four varieties (Amish paste, Cherry, Pear and Early girl). The reason being that the Roma variety has the greatest absorbance and specific activity of arginase. The distribution of Arginase in both seeds and seedlings of *S. lycopersicum* also revers its functionality in many physiological activities involving growth and development.

Based on the result of this research, It is recommended that *Roma* variety should be actively used in plant breeding and hybridization for better vigor of plants of *S. lycopersicum*. Moreover, exogenous arginine should be added to *S.lycopersicum* to enhance the physiological growth and cellular development of the plant.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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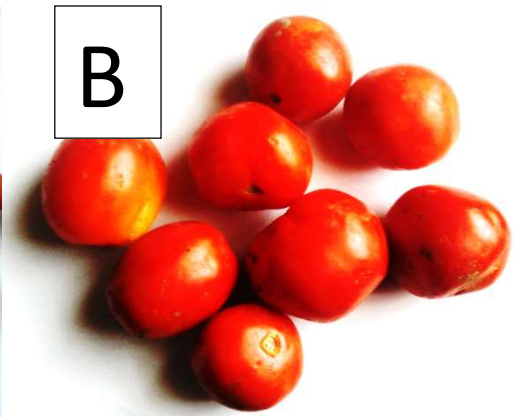
Roma tomato variety



Cherry tomato variety

Pear tomato variety

Amish paste tomato variety

Early girl tomato variety

Plates 1: The five tomato varieties of *Solanumlycopersicum*

Table 1: Quantitative Analysis of Protein in the Five Varieties of *S.lycopersicum*

Tomato Varieties	Absorbance at 450nm
Roma	0.573
Cherry	0.646
Amish paste	0.687
Early girl	0.572
Pear	0.589

Table 2: Quantitative Analysis of Arginase Activity in the Five Varieties of *S.lycopersicum*

Tomato Varieties	Absorbance at 450nm	Arginase SA (µmol/min) in seeds	Absorbance at 450nm	Arginase SA (µmol/min) in seedlings
Roma	0.623	14.38×10^{-3}	0.234	5.4×10^{-3}
Cherry	0.310	7.15×10^{-3}	0.198	4.6×10^{-3}
Amish paste	0.493	11.38×10^{-3}	0.207	4.8×10^{-3}
Early girl	0.584	13.45×10^{-3}	0.135	3.1×10^{-3}
Pear	0.556	12.83×10^{-3}	0.102	2.3×10^{-3}

SA= Specific Activity

Table 3: Germination Percentage of the Five Varieties of Solanumlycopersicum

Tomato Varieties	Percentage yield (%)
Roma	73.0
Cherry	54.5
Amish paste	48.6
Early girl	40.5
Pear	32.4

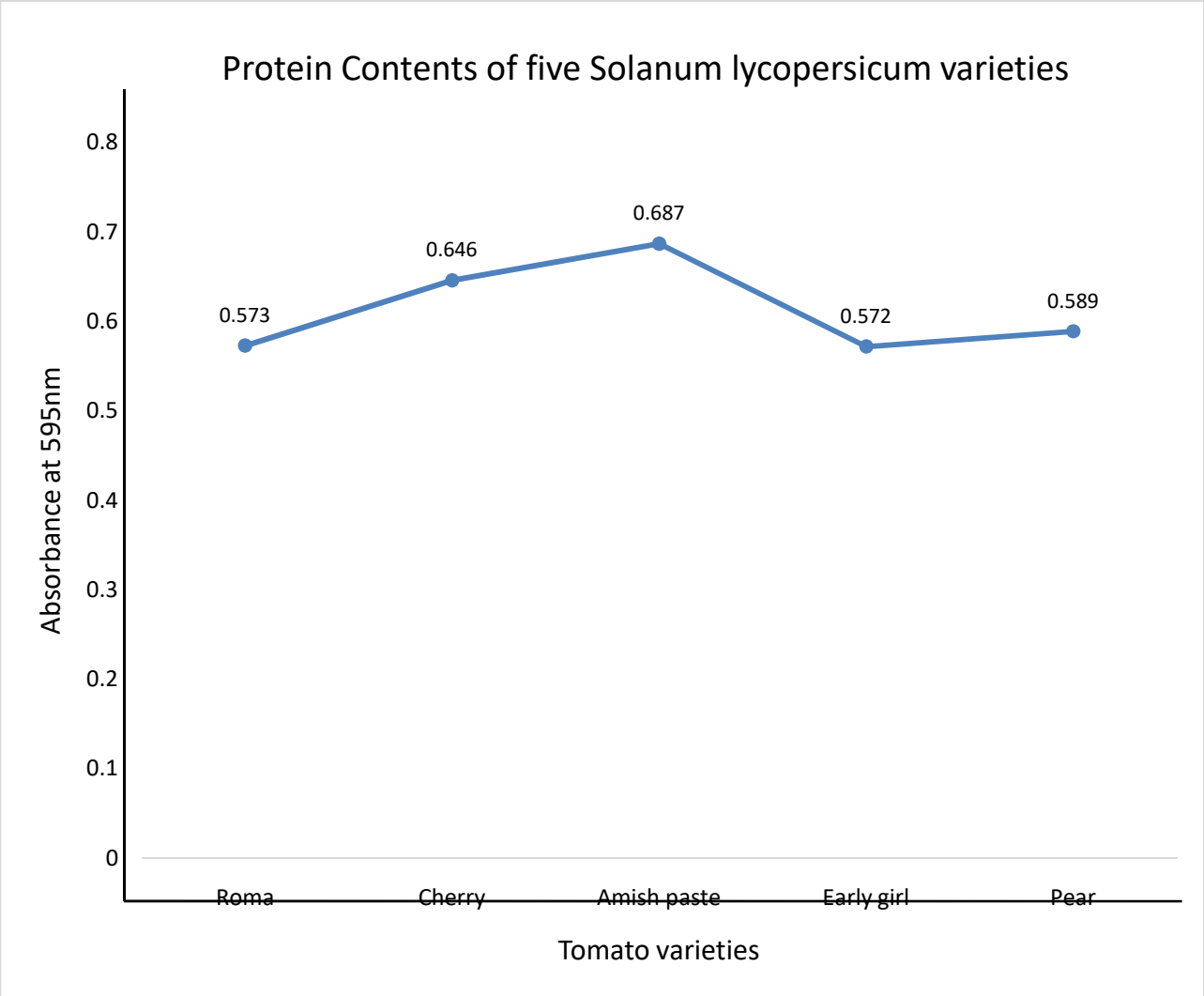


Figure 2: Protein contents of five varieties of *Solanumlycopersicum*.

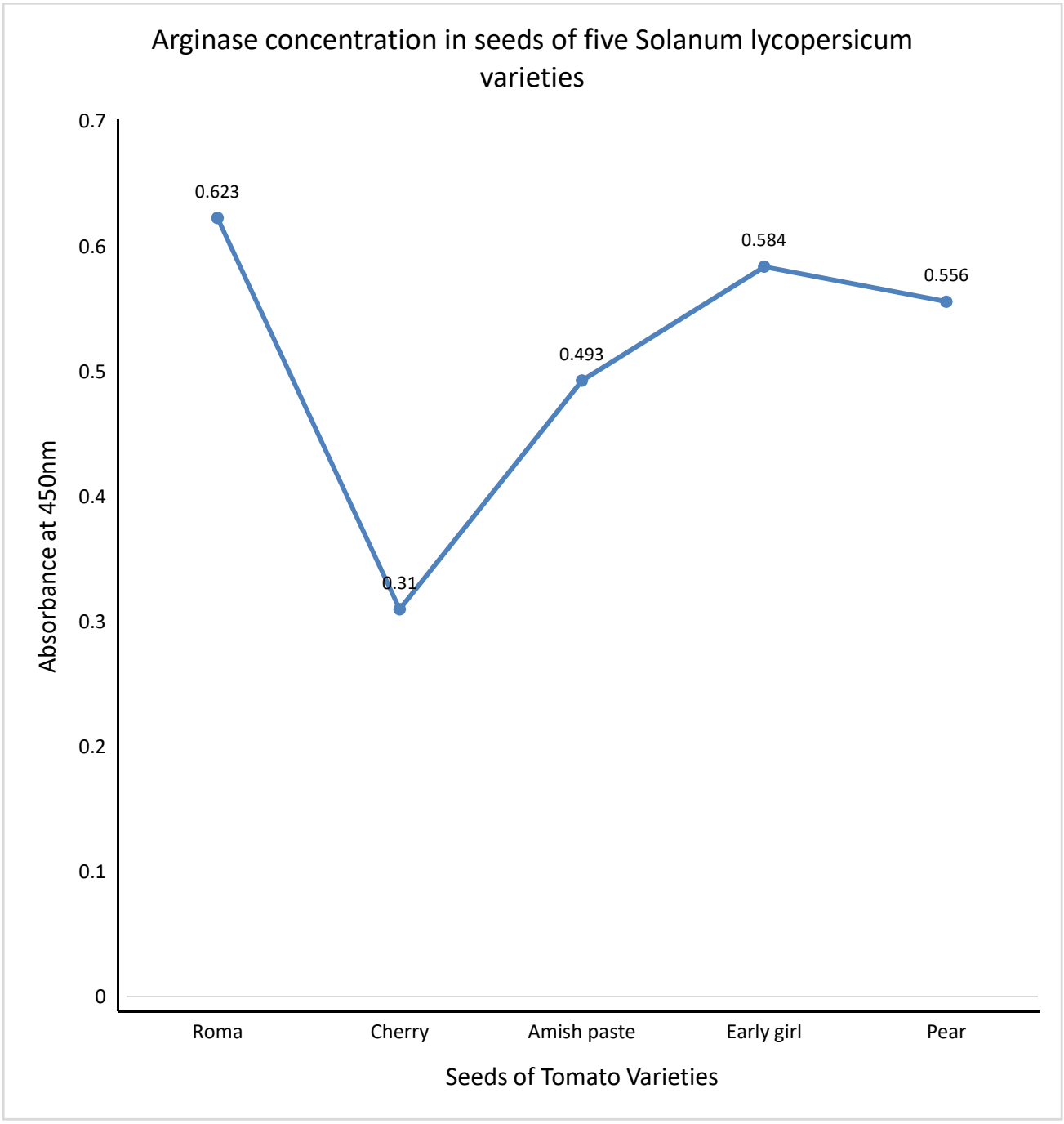


Figure 3: Arginase concentration in the seeds of the five varieties of *Solanumlycopersicum*

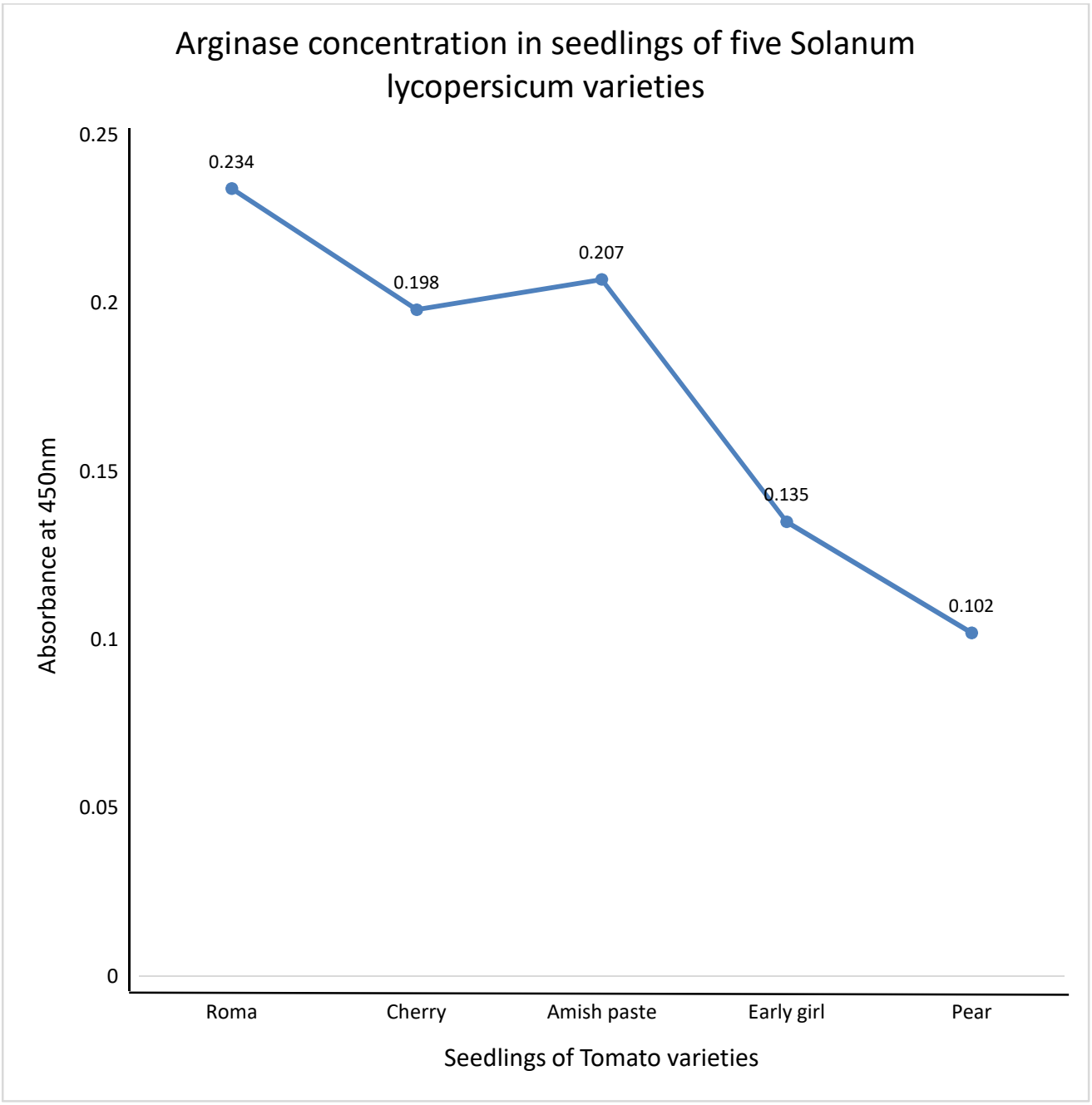


Figure 4: Arginase activities in the seedlings of the five varieties of *Solanumlycopersicum*.

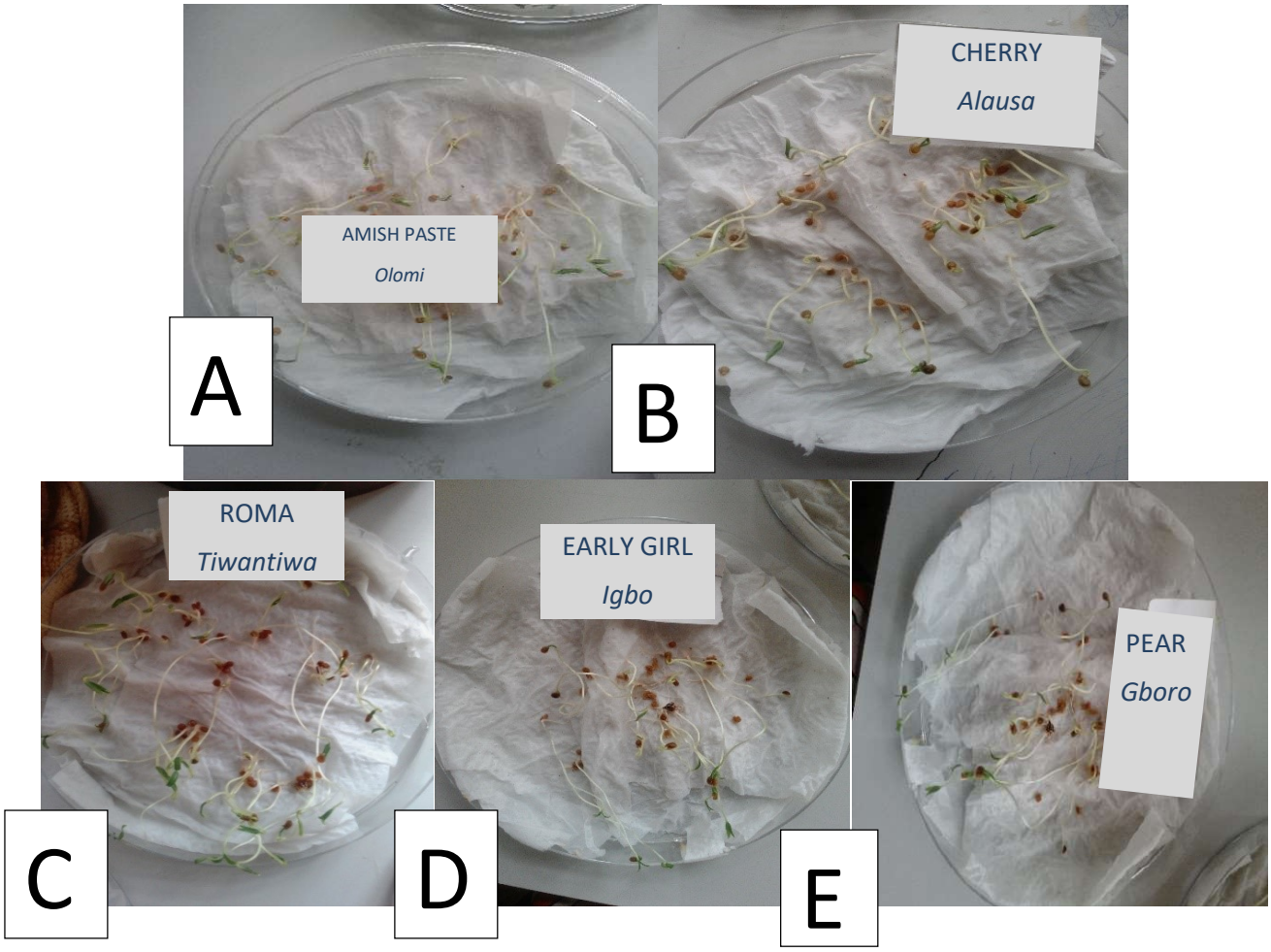


Plate 2: Seedlings of the five varieties of *Solanumlycopersicum*as observed during germination process.

Plate 2	The five tomato varieties' seedlings
A	Amish paste seedlings
B	Cherry seedlings
C	Roma seedlings
D	Early girl seedlings
E	Pear seedlings