Effect of Seed Priming on Specific Activity of Acid Phosphatase and α-Amylase in Germinating Seeds and Correlation Study in Onion (Allium cepa L.)

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ABSTRACT

A field experiment was conducted during 2016-17 and 2017-18 at Instructional Farm, Krishi Vigyan Kendra, Jharsuguda in Guava (Psidium guajava L.) with an objective to evaluate the effect of plant growth regulation practices (both cultural and chemical methods) on vegetative growth, flowering and yield. The soil of the experimental orchard is red laterite and the climatic condition of the region is sub-tropical dry climate having hot and dry summer and mild winter. The experiment consisted of 8 treatments viz., T1: Control; T2: Shoot bending; T3: 10 cm pruning with complete removal of old leaves, in April-May; T4: 50% fruit thinning randomly by hand at an average fruit weight 15-20 g in April-May; T5: Foliar spray of Naphthalene Acetamide (NAD) @ 50 ppm twice at 15 days interval during April-May; T6: Foliar spray of (2, 4-D) @ 60 ppm, twice at 15 days interval in the during April-May; T7: Foliar spray of urea @ 15%, twice at 15 days interval during April-May and T8: Foliar spray of Dinitro Ortho Cresol (DNOC) @ 10ppm, twice at 10 days during April-May. These 8 treatments were evaluated in randomized block design with three replications. From the experiment, it was found that untreated treatment (Control i.e. T1) resulted tallest plant while minimum plant height was recorded in Bending (T2). The canopy spread in East-West and North-South were recorded highest in T3 (10 cm pruning). Number of days required for emergence of new shoot was recorded minimum of (18.00 & 19.2 days) shoot bending (T2) and 10 cm pruning (T3) respectively while it was highest (35.8 days) in the untreated control (T1). Shoot bending (T2) treatment took minimum days (43.5 days) while control plants (T1) took maximum days (50.5days) for initiation of flowers in the new shoots. The period required for fruit maturity varied from 126.7 days in bending (T2) to 134.5 days in control plants (T1). Shoot bending (T2) recorded highest number of fruits per plant during both the years and were significantly different from other treatments. From the mean data heaviest fruit (133.6 g) was obtained in T5 (50 ppm NAD) followed by in T3 (10 cm pruning) and 129.2 g T6 (60 ppm 2,4-D) whereas lowest fruit weight was obtained in control (88 g). Similarly, 50 ppm NAD (T5) gave highest fruit yield (8.80 kg/plant) closely followed by in T3 (10 cm pruning) with a yield of 7.88 kg/plant. The lowest yield was recorded in control (5.43 kg/plant).

Keywords

Onion, Allium cepa L., Seed priming, Acid Phosphatase, α-Amylase, Correlation Germination

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Introduction

The onion seed being small in size requires due cares for sowing in nursery. The unavailability/scanty supply of fresh seed, force farmers to use aged seed for sowing in nursery of which germination is low because of decline in viability. The establishment of seedling is an important factor in bulb production and largely depends on the seed germination and vigour. The pre-sowing seed treatment has been demonstrated to give better seedling under stress conditions like very high or low temperature. To overcome the situation, seed priming is the best option. It is controlled hydration process followed by redrying that permits all metabolic processes but prevent radicle protrusion. Seed priming

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appears to reverse the detrimental effects of seed deterioration. During priming repair of DNA, RNA, protein, membranes and enzymes occurs. Oxygen is also increased, suggesting that respiratory activity is an essential component of repair. Priming increases enzyme activity such as α-amylase activity which breakdown starch stored in seeds to be utilized by growing embryos during germination. Acid phosphatase is believed to be important for phosphorus scavenging and remobilization in plants.

Enzyme helps for many physiological processes, including regulation of phosphorus efficiency. α-amylase plays an important role in hydrolyzing the endosperm starch into sugars, which provide the energy for the growth of roots and shoots (Kaneko et al., 2002). α-amylase hydrolyzes α-1, 4 linkage of starch in a random manner playing an important role in starch metabolism in germinating seed.

Materials and Methods

The study was conducted at Instructional Farm, Krishi Vigyan Kendra, Jharsuguda in Guava (Psidium guajava L.) cv. Arka Mridula during 2016-17 and 2017-18. The soil of the experimental orchard is red laterite and the climatic condition of the region is hot and moist sub humid conditions of Western Central Table Land zone of Odisha.

The guava plants were procured from Central Horticultural Experiment Station, Bhubaneswar. The experiment consisted of 8 treatments (T1: Control, T2: Shoot bending; T3: 10 cm pruning with complete removal of old leaves, in April-May; T4: 50% fruit thinning randomly by hand at an average fruit weight 15-20 g in April-May; T5: Foliar spray of Naphthalene Acetamide (NAD) @ 50 ppm twice at 15 days interval during April-May; T6: Foliar spray of (2, 4-D) @ 60 ppm, twice at 15 days interval in the during April-May; T7: Foliar spray of urea @ 15%, twice at 15 days interval during April-May and T8: Foliar spray of Dinitro Ortho Cresol (DNOC) @ 10ppm, twice at 10 days during April-May). Healthy and disease free lateral shoots were selected for shoot bending with utmost care. Shoot bending was done in such a way that the bent branch did not broken down after bending. Shoots were bent at 90° angle with the help of a piece of rope. Before shoot bending 3-5 leaves were kept at the upper portion of the branch to continue its photosynthesis and respiration process and rest leaves were removed off.

Results and Discussion

Activities of several enzymes associated with the germination process have been proven to change in response to seed priming. These include increases in the activities of acid phosphatase and esterase in lettuce (Khan et al., 1978), α-amylase in rice (Farooq et al., 2006) and antioxidant enzymes in wheat (Afzal et al., 2006). Several enzymes are activated during seed germination including acid phosphatase implicated in the remobilization of phosphorus reserves (Biswas and Cundiff, 1991). Acid phosphatase is believed to be important for phosphorus scavenging and remobilization in plants, also plays important role in many physiological processes, including regulation of phosphorus efficiency.

As per table. 1 and Fig 1 the acid phosphatase activity was found to be higher in fresh untreated seed (0.107) than old untreated seed (0.088). All the treatments in fresh seed lot showed higher activity upto 3rd day of germination than old seed lot. However old seed lot treatments surpassed fresh seed lot treatments on 6th day analysis. In old seed lot treatments the activity increased upto 9th day except in seed priming treatment with Mg 0.75%.
**Table 1** Effect of seed lots and seed priming treatments on specific activity for acid phosphatase (μ moles of p-nitrophenol formed min⁻¹ mg⁻¹ protein) of six hour primed seed, 3 day germinated seed, 6 day and 9 day old seedling

| Treatments   | Sp. act of 6 hr primed seeds | Sp. act of 3 day germinated seed | Sp. act of 6 day old seedling | Sp. act of 9 day old seedling | Sp. act of 12 day germinated seed |
|--------------|-------------------------------|----------------------------------|-------------------------------|-------------------------------|-----------------------------------|
|              | Seed Lot                      | Mean                             | Seed Lot                      | Mean                          | Seed Lot                          |
|              | L₁ (Old)                      | Mean                             | L₁ (Old)                      | Mean                          | L₁ (Old)                          |
| T₁ (Zinc - 0.5%) | 0.101 0.112                  | 0.107                            | 0.171 0.218                  | 0.194                         | 0.80 0.74                        |
| T₂ (Zinc - 0.75%) | 0.094 0.120                  | 0.107                            | 0.172 0.235                  | 0.203                         | 0.83 0.73                        |
| T₃ (Mg - 0.5%)    | 0.098 0.118                  | 0.108                            | 0.181 0.228                  | 0.205                         | 0.77 0.73                        |
| T₄ (Mg - 0.75%)    | 0.114 0.124                  | 0.119                            | 0.188 0.220                  | 0.204                         | 0.77 0.72                        |
| T₅ (Ca - 0.5%)    | 0.107 0.128                  | 0.118                            | 0.177 0.210                  | 0.194                         | 0.81 0.70                        |
| T₆ (Ca - 0.75%)    | 0.129 0.132                  | 0.131                            | 0.197 0.212                  | 0.205                         | 0.79 0.71                        |
| T₇ (GA₃ - 50 ppm) | 0.128 0.121                  | 0.124                            | 0.187 0.211                  | 0.199                         | 0.82 0.72                        |
| T₈ (GA₃ - 100 ppm)| 0.139 0.138                  | 0.138                            | 0.181 0.216                  | 0.199                         | 0.86 0.70                        |
| T₉ (Control)      | 0.088 0.107                  | 0.100                            | 0.179 0.201                  | 0.190                         | 0.63 0.58                        |
| Mean             | 0.111 0.122                  | 0.097                            | 0.181 0.217                  | 0.199                         | 0.79 0.70                        |
| S. E. ±          | Lot Treat LxT                | Lot Treat LxT                    | Lot Treat LxT                | Lot Treat LxT                | Lot Treat LxT                    |
|                 | 0.0004 0.0008                | 0.0012                            | 0.0004 0.0009                | 0.0013                         | 0.0031 0.0066                     |
| C. D.            | 0.0011 0.0023                | 0.0032                            | 0.0012 0.0026                | 0.0037                         | 0.0089 0.0189                     |
Table 2 Effect of seed lots and seed priming treatments on specific activity for α-amylase (µg of maltose released min⁻¹ mg⁻¹ protein) of 6 hr primed seed, 3 day germinated seed, 6 day and 9 day old seedling

| Treatments         | Sp. act of 6 hr primed seeds | Sp. act of 3 day germinated seed | Sp. act of 6 day old seedling | Sp. act of 9 day old seedling | Sp. act of 12 day germinated seed |
|--------------------|-------------------------------|----------------------------------|------------------------------|-------------------------------|----------------------------------|
|                    | Seed Lot                      | Mean                            | Seed Lot                     | Mean                          | Seed Lot                         |
|                    | L₁ (Old)                      | L₂ (Fresh)                      | L₁ (Old)                     | L₂ (Fresh)                    | L₁ (Old)                        |
| T₁ (Zinc - 0.5%)   | 1.27                          | 1.04                            | 1.15                         | 7.28                         | 5.49                            |
|                    |                               |                                 |                              | 6.38                         |                                 |
| T₂ (Zinc - 0.75%)  | 1.00                          | 0.88                            | 0.94                         | 7.08                         | 5.46                            |
|                    |                               |                                 |                              | 6.27                         |                                 |
| T₃ (Mg - 0.5%)     | 1.10                          | 0.87                            | 0.98                         | 7.70                         | 5.37                            |
|                    |                               |                                 |                              | 6.56                         |                                 |
| T₄ (Mg - 0.75%)    | 1.31                          | 1.14                            | 1.22                         | 7.65                         | 5.31                            |
|                    |                               |                                 |                              | 6.50                         |                                 |
| T₅ (Ca - 0.5%)     | 1.38                          | 1.06                            | 1.22                         | 7.14                         | 5.37                            |
|                    |                               |                                 |                              | 6.25                         |                                 |
| T₆ (Ca - 0.75%)    | 1.25                          | 1.11                            | 1.18                         | 7.66                         | 5.44                            |
|                    |                               |                                 |                              | 6.55                         |                                 |
| T₇ (GA₃ - 50 ppm)  | 1.25                          | 1.09                            | 1.17                         | 7.58                         | 5.36                            |
|                    |                               |                                 |                              | 6.47                         |                                 |
| T₈ (GA₃ - 100 ppm) | 1.21                          | 1.04                            | 1.13                         | 7.85                         | 5.39                            |
|                    |                               |                                 |                              | 6.62                         |                                 |
| T₉ (Control)       | 0.90                          | 0.34                            | 0.62                         | 4.91                         | 4.37                            |
|                    |                               |                                 |                              | 4.64                         |                                 |
| Mean               | 1.19                          | 0.95                            | 1.07                         | 7.23                         | 5.28                            |
| S. E. ±            | Lot                           | Treat                           | LxT                          | Lot                          | Treat                           |
|                    | 0.005                         | 0.018                           | 0.0152                       | 0.0267                       | 0.0567                          |
|                    |                               |                                 |                              | 0.0802                       |                                 |
|                    |                               |                                 |                              | 0.0171                       |                                 |
|                    |                               |                                 |                              | 0.0364                       |                                 |
|                    |                               |                                 |                              | 0.0514                       |                                 |
|                    |                               |                                 |                              | 0.0109                       |                                 |
|                    |                               |                                 |                              | 0.0233                       |                                 |
|                    |                               |                                 |                              | 0.033                        |                                 |
|                    |                               |                                 |                              | 0.0109                       |                                 |
|                    |                               |                                 |                              | 0.023                        |                                 |
|                    |                               |                                 |                              | 0.0326                       |                                 |
| C. D.              | 0.0145                        | 0.0309                          | 0.0438                       | 0.0768                       | 0.1629                          |
|                    |                               |                                 |                              | 0.2304                       |                                 |
|                    |                               |                                 |                              | 0.0493                       |                                 |
|                    |                               |                                 |                              | 0.105                        |                                 |
|                    |                               |                                 |                              | 0.1478                       |                                 |
|                    |                               |                                 |                              | 0.0316                       |                                 |
|                    |                               |                                 |                              | 0.067                        |                                 |
|                    |                               |                                 |                              | 0.0948                       |                                 |
|                    |                               |                                 |                              | 0.0312                       |                                 |
|                    |                               |                                 |                              | 0.0662                       |                                 |
|                    |                               |                                 |                              | 0.0934                       |                                 |
Table 3: Initial and final germination (%), seedling vigour index and field emergence (%) as influenced by seed lots and seed priming treatments

| Treatments       | Germination (first count) on 6th day (%) | Final germination on 12th day (%) | Seedling vigour index | Field emergence |
|------------------|------------------------------------------|----------------------------------|-----------------------|-----------------|
|                  | Seed lot                                 | Mean                             | Seed lot              | Mean            | Seed lot       | Mean |
|                  | L₁ (Old)                                 | L₂ (Fresh)                       | L₁ (Old)              | L₂ (Fresh)      | L₁ (Old)       | L₂ (Fresh) |
| T₁ (Zinc - 0.5%) | 72.67                                    | 78.67                            | 80.33                 | 86.67           | 83.50          | 1346.10                  | 1357.40 | 1351.75 | 68.67 | 73.67 | 71.17 |
| T₂ (Zinc - 0.75%)| 72.33                                    | 79.67                            | 80.33                 | 86.00           | 83.17          | 1396.21                  | 1301.72 | 1348.96 | 65.33 | 73.00 | 69.16 |
| T₃ (Magnesium- 0.5%) | 73.67                                | 76.00                            | 81.00                 | 85.33           | 83.17          | 1335.62                  | 1288.53 | 1312.07 | 71.67 | 76.00 | 73.83 |
| T₄ (Magnesium- 0.75%) | 74.67                                | 79.00                            | 80.33                 | 86.67           | 83.50          | 1301.77                  | 1326.45 | 1314.11 | 72.00 | 73.33 | 72.67 |
| T₅ (Calcium - 0.5%) | 71.33                                 | 75.33                            | 80.67                 | 86.33           | 83.50          | 1337.17                  | 1327.05 | 1332.11 | 69.33 | 73.67 | 71.50 |
| T₆ (Calcium - 0.75%) | 70.67                                 | 77.67                            | 81.33                 | 85.33           | 83.33          | 1358.00                  | 1304.03 | 1331.01 | 69.33 | 74.67 | 72.00 |
| T₇ (GA₃ -50 ppm) | 73.67                                    | 78.67                            | 81.00                 | 85.63           | 83.33          | 1330.42                  | 1333.89 | 1332.16 | 73.00 | 74.67 | 73.83 |
| T₈ (GA₃ -100 ppm) | 70.67                                    | 78.67                            | 81.33                 | 85.67           | 83.50          | 1382.23                  | 1340.04 | 1361.13 | 72.67 | 74.33 | 73.50 |
| T₉ (Control)                      | 63.33                                    | 76.33                            | 77.00                 | 84.33           | 80.67          | 1237.70                  | 1284.00 | 1260.85 | 64.00 | 72.00 | 68.00 |
| Mean                          | 71.44                                    | 77.78                            | 80.37                 | 85.78           | 83.07          | 1336.14                  | 1318.12 | 1327.13 | 69.56 | 73.93 | 71.74 |
| S.E.±                         | Lot                                       | Treat                            | LxT                   | Lot                          | Treat         | LxT                        | Lot                          | Treat         | LxT |
|                              | 0.383                                    | 0.813                            | 1.15                  | 0.3155                      | 0.6694        | 0.9466                    | 7.301                        | 15.488        | 21.903 |
|                              | 0.629                                    | 1.334                            | 1.886                 |                             |                |                           |                             |               |     |
**Table 4** Correlation of old and fresh seed lot for acid phosphatase enzyme activity with germination, seedling vigour index and field emergence

| Sr. No. | Particular  | Old seed lot | Fresh seed lot |
|---------|-------------|--------------|----------------|
|         | Germi-      | S.V.I.       | Field       | Germi- | S.V.I.       | Field |
|         | nation.     |              | emergence   |         |              | emergence |
| 1.      | 6 hr. priming | 0.619        | -           | -       | 0.313        | -       |
| 2.      | 3 Days      | 0.386        | -           | -       | 0.692        | -       |
| 3.      | 6 Days      | 0.758        | -           | -       | 0.509        | -       |
| 4.      | 9 Days      | -0.406       | -0.578      | -0.262  | 0.002        | -0.199  | -0.253  |
| 5.      | 12 Days     | -0.947*      | -0.908*     | -0.922* | 0.324        | 0.821   | 0.313   |

**Table 5** Correlation of old and fresh seed lot for α-amylase enzyme activity with germination, seedling vigour index and field emergence

| Sr. No. | Particular  | Old seed lot | Fresh seed lot |
|---------|-------------|--------------|----------------|
|         | Germi-      | S.V.I.       | Field       | Germi- | S.V.I.       | Field |
|         | nation.     |              | emergence   |         |              | emergence |
| 1.      | 6 hr. priming | 0.879*       | -           | -       | 0.935**      | -       |
| 2.      | 3 Days      | 0.904*       | -           | -       | 0.868*       | -       |
| 3.      | 6 Days      | 0.888*       | -           | -       | 0.896*       | -       |
| 4.      | 9 Days      | -0.637       | -0.663      | -0.836  | 0.533        | 0.484   | 0.192   |
| 5.      | 12 Days     | -0.668       | -0.707      | -0.524  | 0.915*       | 0.878   | 0.874   |

**Fig. 1** Acid phosphatase enzyme activity as influenced by seed lots and seed priming treatments
In fresh seed lot treatments the activity increased in Zn 0.5%, Ca 0.75%, GA 50ppm and untreated fresh seed upto 9th day. In rest of the treatments of fresh lot, the activity showed increased trend upto 12th day except in GA 100 ppm in which the activity was same on both days.

The stimulation of phosphatases activities appears to maintain higher cell metabolic status by providing a higher rate of phosphate release and active transport and biosynthetic events in growing embryo axes (Dubey and Sharma, 1990). Nasri et al., (2011) showed that there was significant improvement in acid phosphatase and phytase activities in roots, shoots and cotyledons due to osmopriming treatment. These enzymes activities were greater in primed seeds than in non-primed seeds in saline conditions.

Starch is the principal storage polysaccharide in plant cells. It is made up of about 10-20% of amylose and about 80-90% of amylopectin. α-amylase is the hydrolytic enzyme which hydrolyze starch. α-amylase hydrolyzes α-1, 4 linkage of starch in a random manner playing an important role in starch metabolism in germinating seed.

α-amylase activity in old untreated seed was more (0.90) as compared to fresh untreated seed (0.34). After 6 h seed priming α-amylase activity was more in old seed lot treatments than fresh seed lot treatments. In the case of old seed lot treatments α-amylase activity increased upto 6th day except in old untreated seed wherein it increased upto 9th day. Later on the activity decreased. α-Amylase activity was greater in old seed lot treatments upto 9th day as compared to fresh seed lot treatments. In old seed lot treatments, seed priming treatments with magnesium (0.5% & 0.75%) and calcium (0.5% & 0.75%) recorded comparatively higher α-amylase activity than other treatments (Table 2 & Fig 2). During the germination process, gibberellic acid is released from the embryo and activates some responsible genes of α-amylase mRNA transcription (Taiz and Zeiger, 1998). α-amylase plays an important role in hydrolyzing the endosperm starch into sugars, which provide the energy for the growth of roots and shoots (Kaneko et al., 2002).

Farooq et al., (2012) showed that seed priming in rice improved germination and emergence, allometry, kernel yield, and its quality. The results suggest that physiological changes produced by osmohardening enhanced the starch hydrolysis and made
more sugars available for embryo growth, vigorous seedling production and, later on, improved allometric, kernel yield and quality attributes.

The data presented showed significantly better germination for seed priming with magnesium (0.75%) (76.83%) at first count on 6th day followed by GA3 (50ppm) (76.17%). The differences in germination percentage of seeds appeared to be significantly influenced due to seed treatment (Table 3). The results of present study are in agreements with observations of Basra et al.(1989).

In the case of final germination it was observed that fresh seed lot was significantly superior than old seed lot. The seed priming treatments were non significant. The interaction was also found non-significant. Treatment zinc (0.5%) and magnesium (0.75%) showed highest germination of 86.67% while minimum germination of 77.0% was observed in control. It was noticed that due to seed priming treatments, germination of old seed lot was improved as compared to old control. Priming stimulates many of the metabolic processes involved with the early phases of germination. As part of the germination process have been initiated due to priming, seedlings from primed seed grow faster, grow more vigourously, and perform better in adverse conditions (Basker and Hatton, 1987; Desai et al., 1997).

The highest seedling vigour index of 1396.21 was observed in zinc (0.75%) while the lowest seedling vigour index in control (1237.70). The differences for seedling vigour index were non significant for L1 and L2 seed lot, while the seed priming treatments significantly differed. The seedling vigour was highest for treatment T8 GA3 (100ppm). Corbineau et al., (2000) observed that imbibitions of tomato seeds in PEG results in sharp increases in adenosine triphosphatate (ATP), energy charge (EC) and ATP/ADP (adenosine diphosphatase) ratio. These remain higher in primed seeds even after drying than in unprimed seeds. Positive and non significant correlation up to 6 days of germination was observed in old seed lot for germination. In the case of fresh seed lot positive and non significant correlation was observed for germination.

In the case of old seed lot positive significant correlation for α - amylase up to 6 days of germination was found. In fresh seed lot, significant correlation were observed for α - amylase except at 9 days to germination.

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