

True Shallot Seed production during off season

1 **ACCEPTED MANUSCRIPT**

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6 Putra RE, Ramadan DB, Adin A, Kinasih I, Rosmiati M

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18 **VERNALIZATION AND BENZIL AMINO PURIN APPLICATION UNABLE TO**
19 **ENHANCE TRUE SHALLOT SEED (TSS) PRODUCTION DURING OFF SEASON****

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21 **Ramadhani Eka Putra^{1*}, D Beta Ramadan², Adriyanita Adin³, Ida Kinasih⁴ and Mia**
22 **Rosmiati¹**

23 ¹Agricultural Engineering Study Program, School of Life Sciences and Technology, Institut
24 Teknologi Bandung, Bandung 40132, Indonesia

25 ²Bioresource Management Study Program, School of Life Sciences and Technology, Institut
26 Teknologi Bandung, Bandung 40132, Indonesia

27 ³East-West Seeds, P.O. Box 1, 41181 Campaka, Purwakarta, Indonesia

28 ⁴Departement of Biology, Universitas Islam Negeri Sunan Gunung Djati Bandung, Bandung 40614,
29 Indonesia

30 *Corresponding author, e-mail: ramadhani@sith.itb.ac.id

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32 18-20 October 2017, Serang, Banten, Indonesia
33

34 **ABSTRACT**

35 The application of seed for true shallot cultivation is an alternative of the more common
36 cultivation practice, in which 30% of harvested tubers used for cultivation purposes. The seed
37 production of this temperate tuber, in the tropical region, is quite challenging due to low flowers
38 and seed formation. Several studies showed that vernalization (cold induction) and application of
39 Benzil Amino Purin (BAP) could be applied to improve flowering and seed production. However,
40 such studies were conducted during the best cultivation period for about 3 months and thus, limit
41 the production period of seeds. This study was conducted to observe the effect of both methods
42 outside cultivation periods to flower and capsule numbers, fruit set, and weight of 100 seeds
43 compared with common cultivation. In this study, bulbs of onion vernalized at 10°C for 30 days
44 then became subjected to synthetic hormone (BAP) prior planted while control group The results
45 showed that BAP treated shallot group has the lowest values for all observed parameters (1552.67,
46 312.11, 22.5%, 0.2244 gram) compared to those vernalization treated group (1592.44, 623, 30.5%;
47 0.2261 gram) and control group (6774.67; 3898.44; 57.06%; 0.3304 gram). Based on this study, it
48 could be concluded that common cultivation is a better method to produce true shallot seeds during
49 the offseason.
50

51 **Keywords:** Benzil Amino Purin, True Shallot Seeds, Vernalisasi
52

53 **INTRODUCTION**

54 True shallot (*Allium ascalonicum*) is one of the most important tubers in Indonesia. Market
55 demand for this commodity has increased annually with 5.30% average consumption growth
56 (Kementrian Pertanian 2015). In Indonesia, true shallot farmers usually cultivate this commodity by
57 its vegetative form. This method has several disadvantages, such as (1) short storage period
58 (Suwandi & Himan 1995), (2) inconsistent quality (Balai Penelitian dan Pengembangan Pertanian
59 1995), (3) higher possibility of disease spreading (Permadi 1995), (4) higher production cost (Gina
60 & Rofik 2010), and (5) significant amount of harvested tuber unable to be sold (Permadi &

61 Putrasamedja 1991, Basuki 2009) which prevented total true shallot production to fulfill market
62 demand.

63 To meet the market demand, the improvement of the national shallot production through the
64 use of botanical seeds or true shallot seed (TSS) in shallot cultivation is necessary. The seeds have
65 longer storage time, up to six times of generative form, and nullified the need for large storage room
66 (Basuki 2009) while it also reduces production cost (Permadi & Putrasamedja 1991, Basuki 2009).

67 However, most of the local true shallot growing areas have not applied this method due to
68 the limited amount of seed availability (Rosliani 2013). Environmental factors, such as average
69 temperature, photoperiod, average humidity, are believed to be the limiting factor for seed
70 production in Indonesia (Fahrianty 2013, Wu *et al.* 2015). This biennial plant produces bulb as an
71 overwintering stage of the life cycle and produces flowers in the spring after a period of winter
72 (known as vernalization) (Brewster 2008). Furthermore, true shallot also requires long photoperiod
73 (>12 hours) to ensure flowering and seed production (Kamenetsky & Rabinowich 2001).
74 Vernalization of bulb before planting ensures early flowering of seed crop (Brewster 1994) and
75 produces a heavier yield of seeds (Jones & Mann 1963, Mollah *et al.* 2005, Ami *et al.* 2013) as the
76 result of increasing gibberellin endogen and auxin production (Dinarti *et al.* 2011). Therefore, most
77 true shallot seed producers in Indonesia apply low-temperature shock treatment to the mother bulb
78 and establish the plantation in high land.

79 In order to improve seed production, synthetic plant hormone-like Benzil Amino Purin
80 (BAP) is also applied to mother bulb prior to planting. Some studies reported the positive effect of
81 this substance on flower and seed production (Youngkoo *et al.* 2006, Roslian *et al.* 2012).

82 It is possible to produce true shallot seeds in Indonesia by the application of the
83 vernalization of mother bulb and plantation in the highland, mostly in dry seasons. Present study
84 focused on the possibility and limitation of the application of the vernalization technique and BAP
85 for true shallot seed production during the rainy season. The result of this study may provide
86 valuable information to increase the true shallot seed production through the development of the
87 production system during the rainy season, a less optimum period for production.

88

89

MATERIALS AND METHODS

90 Study area and period

91 The study was conducted at the greenhouse of the East West Seed research station and
92 School of Life Sciences and Technology, Institut Teknologi Bandung. True shallot was cultivated
93 from October 2016 to April 2017 at the East West Seed research station, while the quality of seed
94 produced was assessed at Institut Teknologi Bandung. The field experiment was conducted at rainy

True Shallot Seed production during off season

95 season with the average temperature between 19 to 23°C which is considered as off-season for true
96 shallot seed production.

97

98 **Variety**

99 Variety Bima was used in the research program. It is released by Balai Penelitian Tanaman
100 Sayuran. This variety is considered as the most widely

101

102 **Vernalization of mother bulbs**

103 Bulbs of onion were put in white cotton cloth bags and vernalized in a refrigerator at a
104 calibrated temperature of 10°C for 30 days. After bulbs vernalization, a total of 60 bulbs were
105 subjected to synthetic hormone treatment by dipping them in the BAP solution. While another 60
106 bulbs were stored under controlled temperature ($21\pm 3^\circ\text{C}$) and serve as the untreated control. All
107 bulbs were dipped into fungicide to prevent fungi attack before planted.

108

109 **Land Preparation**

110 The land was thoroughly prepared by plowing and cross plowing followed by laddering. The
111 subsequent operations were done with harrow, spade, hammer etc. Weeds and stumblers were
112 collected and removed from the field. Irrigation and drainage channels were made around the plots.
113 The corners of the plots were trimmed by the spade.

114

115 **Plant spacing**

116 The planting distances between rows and between bulbs were 25 cm and 20 cm,
117 respectively. Each plot contained four rows. Fifteen seed bulbs were sown in each row. a total of
118 180 bulbs were sown at 7 cm depth.

119

120 **Application of fertilizer and cultivation practices**

121 True shallot was fertilized with recommended doses of N:P:K 16:16:16 and dolomite.
122 Watering, weeding, and fungicide applications was conducted once a week during shallot
123 cultivation. During flowering period, flowers were covered with plastic cover to prevent it from the
124 destruction by rainfall. Pollination was conducted by hand pollination.

125

126 **Harvesting and processing**

127 The duration of true shallot cultivation was 115-130 days. When the seeds inside the
128 capsules become black and more than 25% black seeds were exposed on the umbel, each umbel was

129 cut with 5 cm of the flower stalk. Harvesting was conducted on day 116, 123, and 130. The umbels
130 were sun-dried. Threshing was done by light beating and hand rubbing of the umbels. The seeds
131 were cleaned and sun-dried up to 7 days until seed moisture reduced to below 8%. The seeds of
132 each harvest were processed separately and contained in a separate paper bag and preserved for
133 further use (Mollah *et al.* 2015).

134

135 **Weight of 100 seeds**

136 One hundred seeds were selected at random from each harvest. The 100-seed weight was
137 recorded on an electric balance and expressed in gram (g).

138

139 **Seed germination**

140 Germination test was carried out in a plastic tray according to the International Rules for
141 Seed Testing (ISTA, 1996). A paper towel dipped in liquid fertilizer was used as a substrate for the
142 germination test. The plastic tray was filled with a moist paper towel. Adequate moisture was
143 maintained in the substrate. One hundred seeds were taken at random and placed in the tray. The
144 number of normal seedlings, abnormal seedlings, dead seeds, and ungerminated seeds was counted
145 for two weeks. Germination percentage was determined by the following formula.

146

$$147 \text{ Germination} = [(\text{Number of seedlings} / \text{Number of seeds tested})] \times 100\% \quad (1)$$

148

149 **Data Analysis**

150 The normality of data was analyzed by One-Sample Kolmogorov-Smirnov. The differences
151 among treatment on flowering initiation period, flower numbers, capsule numbers, fruit set, seed
152 numbers, weight on 100 seeds, and seeding rates were analyzed by one-way ANOVA with a
153 significant level of $P < 0,05$. Tukey analysis was conducted as the post hoc test when ANOVA
154 showed significant value. All analyses were conducted by SPSS 16.0.

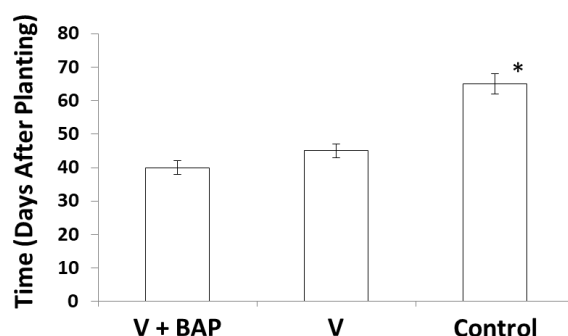
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RESULTS AND DISCUSSION

157 **Flowering initiation**

158 The time required to produce flowers in untreated shallot plants (control group) was
159 significantly longer than those in other groups. While those in V+BAP and V groups were relatively
160 similar (Fig. 1).



161

162 Figure 1 Time required for producing flowers among all treatments. V + BAP = Vernalization +
 163 Benzil Amino Purin (BAP) treatment, V = Only vernalization. (*) significant at $P < 0.05$

164

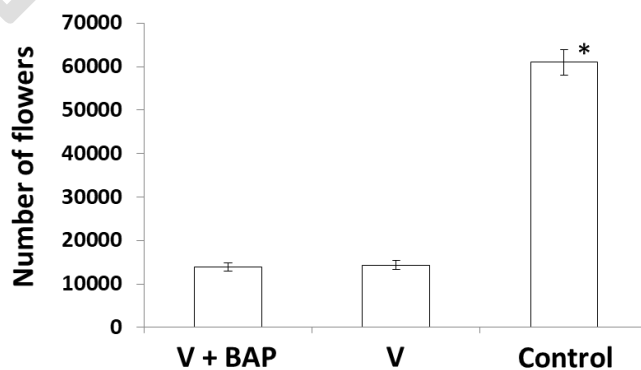
165 This result is in accordance with previous studies which revealed that vernalization
 166 treatment on shallot bulbs required shorter time to produce flowers (Satjadiputra 1990, Yan *et al.*
 167 2003, Islam *et al.* 2010, Andres and Coupland 2012, Fahrianty 2013, Ream *et al.* 2013, Wu *et al.*
 168 2015). The result showed the importance of vernalization treatment to initiate flowering which
 169 might relate to the temperate origin of true shallot. It is reported that vernalization blocked
 170 flowering repressor and induced expression of genes responsible for the flowering (florigen) (Lee *et*
 171 *al.* 2013). Vernalization could also promote the up-regulation of some key cytokinin signaling
 172 regulators which induced flowering (Wen *et al.* 2017). Application of BAP, a cytokinin synthetic,
 173 might induce gibberellin signaling that reduced flowering initiation time (Tarkowska 2012, Wong,
 174 *et al.* 2013).

175

176 **Number of flowers, capsules, and seeds produced**

177 The control groups produced significantly more flowers than other groups. On the other
 178 hand, the number of flowers produced by both V+BAP and V groups was relatively similar (Fig. 2).

179



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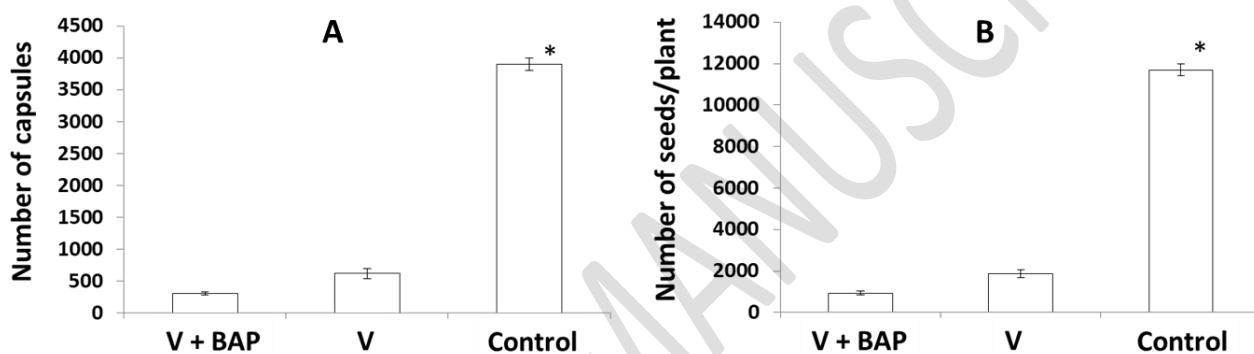
181 Figure 2 Number of true shallot flowers among treatments. V + BAP = Vernalization + Benzil
 182 Amino Purin (BAP) treatment, V = Only vernalization. (*) significant at $P < 0.05$

183

184 The previous study (Fahrianty, 2013) reported the positive effect of vernalization on flower
 185 initiation and the number of flowers produced by shallot. However, the study was conducted on dry
 186 season, the best season for true shallot seed production. The lower number of flowers produced by
 187 vernalization groups in this study could be related to insufficient photoperiod. The true shallot is a
 188 long day plant that required 12 hours light period (Currah & Proctor 1990). Shorter and epileptic
 189 light conditions during the rainy season may negate the positive effect of vernalization on flower
 190 production due to less optimal photoperiod (Dennish & Peacock 2009, Wu *et al.* 2015).

191 Lower numbers of flowers resulted in fewer number of capsules (Fig. 3A) and seeds (Fig.
 192 3B) of vernalization treated shallot groups. Most of the seed losses were caused by flower abortion
 193 and infection by fungi on the flowers of vernalization groups.

194



195

196 Figure 3 (A) Number of true shallot capsules and (B) Seeds per plant among treatments.
 197 V + BAP = Vernalization + Benzil Amino Purin (BAP) treatment, V = Only vernalization. (*)
 198 significant at $P < 0.05$.

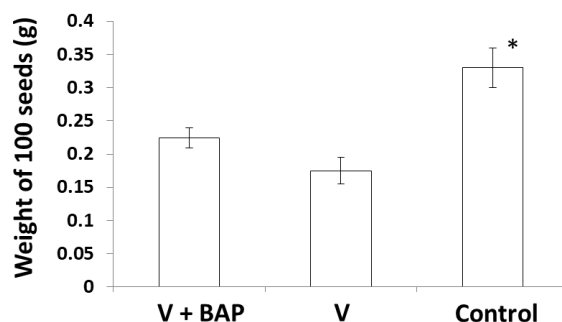
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200 The result might indicate plants of vernalization groups had lower resistance to diseases due
 201 to high humidity. Vernalization reduced vegetative period which benefited the seed production,
 202 when plant growth in optimum condition, as most energy produced by plant could be fully used to
 203 seed production. However, under sub-optimal conditions, the plant had to overcome environmental
 204 stress and allocated less energy to seed production. On the other hand, a longer growing period of
 205 the control group could increase seed yield as also reported by Farghali (1995), possibly through
 206 more energy available for seed production.

207

208 **Seeds Quality**

209 The weight of 100-seeds of the control groups was significantly higher than that of other
 210 groups, while the V+BAP group produced slightly heavier seeds than the V group (Fig. 4).



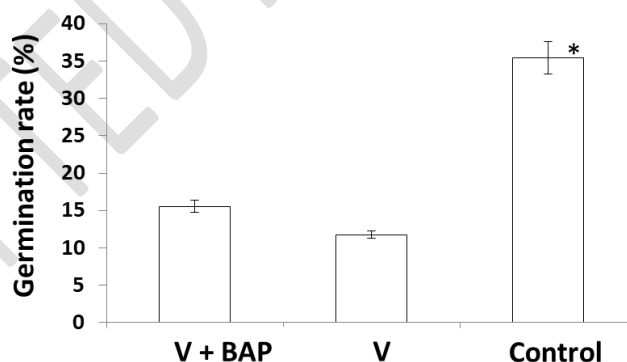
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212 Figure 4 Weight of 100-seeds among treatments. V + BAP = Vernalization + Benzil Amino Purin
213 (BAP) treatment, V = Only vernalization. (*) significant at $P < 0.05$.

214

215 The results of the seed weight of vernalization groups were on the the contrary with that
216 found in previous studies that indicated the positive effect of vernalization on seed weight (Mollah
217 *et al.* 2005, Ami *et al.* 2013). The high humidity of the rainy season might cause significant damage
218 to the seed that reduced its weight (Ku *et al.* 2008). The addition of BAP after vernalization
219 improved the seed weight as it could induce cell growth and tissue differentiation (Rosliani 2013).
220 Based on this study, it could be hypothesized that vernalization only induces flower while the seed
221 quality depends on different mechanisms such as the effect of vegetative propagation, flower
222 numbers and the availability of the pollinator (Krontal *et al.* 2000). Therefore, further study is
223 necessary to test this hypothesis.

224



225

226 Figure 5 Germination rate of seeds produced among treatments. V + BAP = Vernalization + Benzil
227 Amino Purin (BAP) treatment, V = Only vernalization. (*) significant at $P < 0.05$.

228

229 Germination rate of seeds produced by control groups were significantly higher than that of
230 other groups, followed by V+BAP group and V group (Fig. 5). The germination rate of seed highly
231 depends on seed weight which explained low germination rate of vernalization groups (Gamiely *et*
232 *al.* 1990, Mollah *et al.* 2005). The germination rate of seed recorded in this study also much lower
233 than those produced in optimal season which indicated the importance of planting date (Mollah *et*
234 *al.* 2005, El-Helaly & Karam 2012).

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236

CONCLUSION

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ACKNOWLEDGEMENTS

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