

# JKSUS Plagiarism Report

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1 **Deciphering the role of exogenously-applied vanillic acid in regulating drought stress**  
2 **tolerance in pea (*Pisum sativum* L.): key growth and physio-biochemical attributes**

3

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14 **Short title:** Vanillic Acid Enhances Drought Tolerance in Pea Plants

15 **Declarations** <sup>10</sup>

16 **Conflicts of interest/Competing interests**

17 The authors declare no conflict of interest.

18 **Ethics approval**

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20 **Consent to participate**

21 All authors consent to participate in the manuscript publication

22 **Consent for publication**

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33 **Author Contributions**

34 Conceptualization and methodology, AR, NAA and MA. visualization and supervision, N.A.A.;  
35 writing, M.A.; data analysis and review and editing, AAA and PA. All authors have read and  
36 agreed to the published version of the manuscript.

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38

### 39 **Abstract**

40 To investigate the impact of exogenously applied vanillic acid (VA) in mitigating the adverse  
41 effects of drought stress, a greenhouse experiment was conducted on pea plants (*Pisum sativum*  
42 L.). The pea seeds were primed for 14 h in varying concentrations (0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0,  
43 and 6.0 mM) of VA Then, thirty-five days old pea plants were subjected to control (100% field  
44 capacity) and water deficit conditions (60% F.C.). After thirty days of water stress treatments, the  
45 data showed a notable reduction in shoot and root fresh and dry weights, shoot and root lengths,  
46 and chlorophyll contents. While, water deficit stress led to an increase in leaf free proline, total  
47 phenolics, glycinebetaine (GB), ascorbic acid AsA) as well as the activities of catalase (CAT),  
48 superoxide dismutase (SOD) and peroxidase (POD) enzymes. We observed that seed priming with  
49 various concentrations of vanillic acid resulted in significant enhancement in shoot and root fresh  
50 and dry weights, shoot and root lengths, chlorophyll contents, proline, total phenolics, GB, AsA  
51 and the activities of POD, SOD and CAT enzymes of pea plants. Of varying concentrations of VA,  
52 2.0 and 4.0 mM VA were more effective in improving the plant morphology and physio-  
53 biochemical metabolites of pea plants. So the results of the present study suggested that the  
54 improvement in growth and different physio-biochemical characteristics can be attributed to the  
55 VA-induced upregulation of osmoprotection and oxidative defense system of pea plants.

56 **Keywords:** Water stress; Pea (*Pisum sativum* L.); vanillic acid; antioxidants; osmoprotectants

### 1 **Introduction**

57 Often, the term "drought" refers to shortage of water compared to the demand of the plant  
58 according to the prevailing environmental conditions (Ali et al., 2016). Several regions of the  
59 world are currently experiencing drought stress due to unpredictable climate change (Javed et al.,  
60 2016; Lamaoui et al., 2018; Naumann et al., 2018; Seleiman et al., 2021). The drought-prone

61 regions are increasing due to improper use of irrigations, so the crop productivity on such lands is  
62 hampering significantly (Naumann et al., 2018).

63 The condition of water shortage causes a significant reduction in leaf mass and area with a  
64 significant change in other morphological traits, such as the number of leaves per plant, and plant  
65 height (Seleiman et al., 2021; Yang et al., 2021). Plant developmental processes significantly slow  
66 down as drought duration and intensity increase (Duan et al., 2017). Moreover, during heat and  
67 drought stress, metabolic pathways experience significant alterations (Naz et al., 2014; Akram et  
68 al., 2016), and differentially regulated metabolism-related gene expression can be found,  
69 particularly in the cellular organelles such as mitochondria and plastids (Oleti, 2018). Water  
70 shortage has a significant impact on the functioning of a variety of metabolic processes, including  
71 the rate of photosynthetic activity, source-sink transport, and seed production (Aslam et al., 2013;  
72 Sehgal et al., 2018). For example, excessive generation of reactive oxygen species (ROS)  
73 generated by water stress deteriorates the chloroplast membranes, as the lipid peroxidation of  
74 cellular membranes is the result of over-production of ROS (Ashraf, 2009). In response to ROS, a  
75 variety of antioxidants (enzymatic/non-enzymatic) accumulate in the cells to reduce the destructive  
76 properties of ROS ((Razzaq et al., 2017; Koşar et al., 2022).

77 Pea (*Pisum sativum* L.) is an economical vegetable crop used all over the world. It is a very  
78 common model for genetic and physiological research. Pea has been the subject of countless  
79 scientific studies due to its simplicity of production, quick generation cycle, and significant  
80 morphological variation (Smykal et al., 2012; Santos et al., 2019). However, it is categorized as a  
81 very sensitive crop in terms of its stress tolerance (Cernay et al., 2015; Devi et al., 2023). Although  
82 a variety of strategies are in vogue to enhance stress tolerance in plants, exogenous application of  
83 inorganic and organic chemicals has shown a promise in terms of improving plant stress tolerance.  
84 Thus, in the present investigation, vanillic acid (VA), one of the potential organic growth  
85 substances, was supplemented to drought-stressed pea plants to examine if this chemical could  
86 improve pea plants' growth under water deficit conditions. Vanillic acid is de-scribed as a  
87 derivative of benzoic acid and an oxidized variant of vanillin is commonly used as a flavoring  
88 agent (Kim et al., 2010). It is a conjugated acid of vanillate and is a naturally occurring phenolic  
89 acid; vanillin is used widely in pharmaceuticals, cosmetics and the food industry as a flavoring  
90 agent (Imming et al., 2006). It was first reported in *Melilotus messanensis* (Macías et al., 1997),  
91 then in *Chenopodium murale* (Batish et al., 2007) and *Dactylis glomerata* (Parveen et al., 2011).

92 Being a phenolic compound, vanillic acid in plants reduces the activity of root system by the  
93 allelopathy phenomenon (Chen et al., 2011). Different fruits (grapes, pomegranate, etc.), herbs and  
94 spices (cinnamon, tea, rosemary, sage, thyme, oregano, mint, ginger, etc.) and vegetables  
95 (pumpkin, broccoli, drumstick, curry, nettle, etc.) are the main source of vanillic acid (Ingole et  
96 al., 2021).

97 The influence of vanillic acid on the development and metabolism of different plants has  
98 been examined in different studies. For example, exogenously applied vanillic acid promoted  
99 alfalfa seedlings' growth, particularly the development of plant aerial components (Khaleda et al.,  
100 2017). Likewise, another study, (Parvin et al., 2020) while assessing the impact of VA on tomato  
101 seedlings under saline stress, found a significant enhancement in growth of the tomato seedlings  
102 which was reported to be associated with reduced stress-induced oxidative stress as well as tissue  
103  $\text{Na}^+/\text{K}^+$  ratio. In the same crop, a marked increase in the actions of key antioxidative enzymes was  
104 observed (Ghareib et al., 2010; Singh et al., 2021). Moreover, working with maize (Stingu et al.,  
105 2011) showed 45% improvement in the growth of maize seedlings supplied exogenously with  
106 vanillic acid. In pea particularly, the growth of the roots was suppressed by vanillic acid at high  
107 concentrations (Vaughan and Ord, 1990).

108 Keeping in view the effectivity of VA in improving plant metabolism and stress tolerance,  
109 it was hypothesized that seeds treated with different levels of VA might improve the drought stress  
110 tolerance of pea plants. Thus, the primary aim of this study was to evaluate, that up to what extent  
111 exogenously applied vanillic acid as a seed treatment could improve the growth and vital  
112 physiological and biochemical processes in pea.

36

## 2 Materials and Methods

### 2.1 Growth conditions and treatments

114 An experiment using plastic pots (diameter 28.5 cm<sup>2</sup>) containing 7.5 kg soil/pot sandy-clay-  
115 loam (45:25:30) was performed from October to December 2021 to determine the effectivity of  
116 different levels of vanillic acid (VA) in the regulation of drought stress tolerance. The soil had  
117 organic matter, 0.79%; pH 7.9, EC 3.01 dS m<sup>-1</sup>; P, 5.2 mg kg<sup>-1</sup>, and K, 398 mg kg<sup>-1</sup>. The experiment  
118 was designed using a completely randomized approach, involving three factor-factorial [drought

119 (2) x cultivars (1) x levels of VA (8)] with four pots or replications of each treatment (total eight  
120 pots and 40 plants per treatment). The sample size (pots) was a total of 64 (2 x 1 x 8 x 4 = 64)  
121 experimental units. The pots were placed in the Plant Sanctuary, Government College University  
122 Faisalabad, Pakistan with a latitude of 31°O- 26' N; a longitude of 73°O- 06' E and an altitude of  
123 184.4 m. The seed of a pea variety, Sarsabaz, was procured from the Vegetable Section at the Ayub  
124 Agricultural Research Institute in Faisalabad, Pakistan. During the experimental period, the  
125 average (day + night) temperature was 26.8 °C, the average relative humidity was 68.9%, the  
126 average rainfall was 0.3 mm and the average light period was 8.1 h/day. The seeds were surface  
127 sterilized by washing them in 0.05% sodium hypochlorite. Then the seeds were primed for 14 h  
128 with varied levels (0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mM) of VA (C<sub>8</sub>H<sub>8</sub>O<sub>4</sub>; molecular weight,  
129 168.15 g; Across Organic Chemicals, Pakistan). Various concentrations of VA were formulated  
130 using deionized water. Then, 10 seeds were planted in each pot (replicate). Seed germination was  
131 noted daily and all seeds germinated in eight days. Following 15 days of seed germination, a  
132 thinning process was carried out, and each pot/replicate contained five seedlings of uniform size.  
133 At the same time, drought stress treatments comprising a control group (maintained at 100% field  
134 capacity) and a water deficit stress group (subjected to 60% field capacity) were initiated, and the  
135 required levels were maintained based on the soil saturation (32 mL/100 g oven dry soil) level  
136 percentage as well as field capacity (16 mL/100 g dry soil). These levels took twenty days to attain  
137 the desired field capacities (100% and 60%). After thirty days of drought stress initiation, data for  
138 growth and physiological and biochemical characteristics were determined.

## 139 2.2 Morphological attributes

140 Two plants were gently pulled out from each pot/replicate (eight plants per treatment),  
141 followed by thorough rinsing with distilled water and keeping them on a blotting paper to remove  
142 water. The plant samples were separated into roots and shoots, and their lengths were measured.  
143 Subsequently, their fresh weights were recorded. After that, the shoot and root samples were air-  
144 dried before placing them in an oven set at 70 °C for 72 h, and finally, their dry weights were  
145 recorded.

### 146 2.2.1 Leaf relative water contents (LRWC)

147 A fresh leaf 2<sup>nd</sup> one from the top was sampled from the plants and placed in a water tub after  
148 determining their fresh weights. After keeping these samples in water for three hours, their turgid

149 measurements of weights were documented. Then all <sup>59</sup> samples were oven-dried for 72 h and  
150 recorded <sup>21</sup> their dehydrated masses. Following Jones and Turner (1978), RWC was determined.

### 151 2.2.2 Relative membrane permeability (RMP)

152 A young 2nd leaf from the top (500 mg) was shredded in ten milliliters of deionized water.  
153 After two hours, EC<sub>0</sub> was recorded. The specimens were retained for a night and documented their  
154 EC<sub>1</sub>. Then all samples were autoclaved for <sup>30</sup> minutes. The specimens were allowed to cool down  
155 to ambient temperature, and then their EC<sub>2</sub> <sup>54</sup> was measured using a formula <sup>63</sup> proposed by Yang et al.  
156 (1996), and <sup>73</sup> the RMP <sup>18</sup> was calculated.

### 157 2.3 Chlorophyll (a and b) content

158 Following the procedure established by Arnon (1949), a freshly harvested 2nd leaf weighing  
159 <sup>21</sup> 0.5 g was macerated in a mortar and pestle with 10 ml of 80% (v/v) acetone un-der cold conditions.  
160 <sup>73</sup> The samples were kept at 4 °C for 24 h. Subsequently, <sup>63</sup> a spectrophotometer was employed to  
161 <sup>85</sup> measure the absorbance at wavelengths of 645 and 663 nm.

### 162 2.4 Osmoprotectants (Proline and Glycinebetaine)

163 To measure the proline levels <sup>77</sup> following the procedure <sup>85</sup> of Bates et al. (1973), 10 mL of  
164 <sup>22</sup> sulfosalicylic acid (3%) was used to homogenize 0.5 g of fresh young leaf and then filtered. The  
165 sample extract (2 mL) was mixed with acidic ninhydrin (2 ml) and glacial acetic acid (2 ml). Every  
166 sample was subjected to boiling <sup>80</sup> in a water bath, and <sup>18</sup> subsequently, the mixture was placed in an  
167 <sup>30</sup> ice bath. Subsequently, 4 mL of toluene was added to each sample, and <sup>7</sup> the absorbance of the upper  
168 layer was observed at 520 nm.

169 For GB <sup>43</sup> content determination, a fresh young leaf (500 mg) was mixed in 10 mL of deionized  
170 <sup>7</sup> water, and the samples were prepared following the procedure as delineated by Grieve and Grattan  
171 (1983). A spectrophotometer was utilized to record <sup>43</sup> the absorbance of the lower organic layer, at a  
172 <sup>7</sup> wavelength of 365 nm.

### 173 2.5.1 Ascorbic acid content

174 The youngest 2nd leaf from top (500 mg) was homogenized in trichloroacetic acid (10 ml;  
175 6%). Further reactions were carried out following the procedure proposed by Mukherjee and  
176 <sup>53</sup> Choudhuri (1983). A volume of 2 mL of each leaf extract was <sup>93</sup> combined with 2 mL of 2% (v/v)  
177 <sup>27</sup> dinitrophenyl hydrazine. To this mixture, 1.0 mL of 10% (w/v) thiourea was introduced, and the  
178 samples were subjected to boiling <sup>27</sup> in a water bath for 15 minutes before being <sup>27</sup> cooled to room

179 temperature. Following this, 5 mL of 80% (v/v) H<sub>2</sub>SO<sub>4</sub> was added, and the absorbance was  
180 measured at 530 nm.

#### 181 2.5.2 Total phenolic content

182 Freshly harvested top 2nd leaf (250 mg) was extracted in 5 mL (80%) acetone according to  
183 the method developed by Julkunen-Tiitto (1985). Following the centrifugation process, 0.1 mL of  
184 the sample was blended with 2 ml dH<sub>2</sub>O. The sample extract was supplemented with 1.0 mL of  
185 Folin–Ciocalteu's phenol reagent and 5 mL of 20% sodium carbonate. Subsequently,  
186 spectrophotometric analysis was conducted at 750 nm to determine the total phenolic content.

#### 187 2.6 Activities of enzymatic antioxidants

188 A fresh top 2nd leaf was preserved in an ultra-low freezer for a week. Then, a 500 mg leaf  
189 sample was triturated in a K-buffer (10 ml; 50 mM; pH 7.8). After centrifugation, the mixture was  
190 stored in sterilized Eppendorf tubes for determining the activities of POD, CAT and SOD enzymes.  
191 The SOD activity was assessed according to the method described by Giannopolitis and Ries  
192 (1977), whereas those of POD and CAT enzymes were observed using the protocol outlined by  
193 Chance and Maehly (1955).

#### 194 2.7 Statistical analysis

195 The data of different attributes mentioned earlier were subjected to data analysis using Co-  
196 Stat v. 306, employing analysis of variance (ANOVA) in a completely randomized design. Mean  
197 values were subsequently compared using the least significant difference at the 5% probability  
198 level.

### 199 3 Results

200 Drought stress [60% field capacity (F.C.)] significantly ( $P \leq 0.001$ ) inhibited the shoot fresh  
201 (29.9%) and dry weights (28.6%) of pea (*Pisum sativum* L.) plants. However, seed priming with  
202 varying levels (0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mM) of vanillic acid (VA) caused a substantial  
203 ( $P \leq 0.001$ ) improvement regarding fresh and dry weights of pea plant shoots (Table 1; Fig. 1AB).  
204 In general, the levels of 4.0 mM and 6.0 mM of VA exhibited greater effectiveness in promoting  
205 both fresh and dry shoot weights of pea plants both under normal (100% F.C.) and drought stress  
206 (60% F.C.) conditions. Additionally, water stress led to a notable reduction in the fresh ( $P \leq 0.01$ ;  
207 15.5%) and dry ( $P \leq 0.01$ ; 11.23%) weights of roots of pea plants. The influence of VA was also  
208 significantly ( $P \leq 0.001$ ) effective in enhancing root biomass. The more prominent results were



209 found at 2.0 mM and 4.0 mM concentrations, particularly under water deficit conditions.  
210 Exogenous VA demonstrated a significant increase in the fresh and dry weights of the roots of pea  
211 plants (Fig. 1CD).

212 Considerable reductions were observed in both shoot and root lengths of the pea plants ( $P \leq$   
213 0.001) in dehydrated circumstances. Varying concentrations of VA significantly increased the  
214 shoot and root lengths ( $P \leq 0.001$ ) of the pea plants (Table 1). Of all levels of VA used, 2.0 mM  
215 and 4.0 mM VA were more effective in enhancing the shoot and root lengths of the pea plants in  
216 stress environments (Fig. 1EF). Similarly, a marked ( $P \leq 0.01$ ) reduction was detected in the leaf  
217 area of the pea plants (Table 1) under drought stress conditions (Fig. 1G). Varying levels of VA  
218 noticeably ( $P \leq 0.001$ ) enhanced leaf area per plant under both stressed and unstressed conditions.  
219 Of all VA concentrations, 4.0 mM VA was more effective in minimizing the influence of shortage  
220 of water on the pea plants.

221 Under water deficiency, the leaf relative water contents (LRWC) of the pea plants were  
222 recorded to be markedly declined ( $P \leq 0.001$ ). Different VA levels had noticeable effects in  
223 enhancing the LRWC, and of all VA levels, 3 and 6 mM VA were more effective than the other  
224 levels under both normal and water scarcity surroundings (Table 1; Fig. 2A).

225 Under water-deficit stress, relative membrane permeability (RMP) was increased noticeably  
226 ( $P \leq 0.001$ , Table 1) in the pea plants. However, the application of VA decreased the RMP  
227 significantly ( $P \leq 0.001$ ) in the pea plants. Of all VA levels, 6 mM was more effective in dropping  
228 RMP under both water regimes (Table 1; Fig. 2B).

229 Chlorophyll a and total chlorophyll concentrations reduced markedly ( $P \leq 0.05$ ) in water-  
230 stressed circumstances around the pea plants. Nonetheless, there was no noticeable alteration  
231 induced by drought in the chlorophyll b levels and the chlorophyll a/b ratio of the pea plants. The  
232 pre-treatment of seeds with different concentrations of VA significantly enhanced the levels of  
233 chlorophyll a, b, and total chlorophyll ( $P \leq 0.05$ ,  $P \leq 0.001$ , and  $P \leq 0.05$ , respectively). Notably,  
234 among all the levels employed under both water conditions, 2 mM VA exhibited the most  
235 pronounced effectiveness (refer to Table 1 and Fig. 2CDEF). There was no notable alteration  
236 detected in the chlorophyll a/b ratio of the pea plants at both water regimes.

237 A promising ( $P \leq 0.01$ ) increase in proline contents was noticed in shortage of water  
238 situations (Fig. 2G). Seed priming with different levels of VA (0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0  
239 mM), some levels of VA was found to be very effective ( $P \leq 0.001$ ) in enhancing the proline

240 accumulation in the pea plants under water scarcity circumstances (Table 1). Of all concentrations  
241 of VA, 1.0 and 5.0 mM were observed more promising for the pea plants in accumulating a  
242 substantial amount of proline under water deficit stress.

243 A noticeable ( $P \leq 0.001$ ) rise in glycinebetaine (GB) was perceived in drought-stressed pea  
244 plants. Exogenous supplementation of VA was found instrumental for raising GB contents of the  
245 pea plants 4.0 and 6.0 mM doses of VA were rated as the best for achieving increased GB content  
246 in the pea plants at 60% F.C. (Table 1; Fig. 2H).

247 Ascorbic acid (AsA) concentration was recorded to be <sup>76</sup>higher in the drought-stressed pea  
248 <sup>76</sup>plants than in the control untreated plants. However, varying levels of VA considerably ( $P \leq 0.001$ )  
249 improved the AsA contents, and 2.0 mM was more promising than the other VA levels in  
250 increasing the AsA levels in the drought-stressed pea plants (Fig. 3A).

251 Total phenolic contents were significantly ( $P \leq 0.001$ ) higher <sup>61</sup>in the water-stressed pea plants  
252 <sup>61</sup>than those in the untreated plants. The supplementation of VA resulted in a marked rise in the total  
253 phenolic content of the pea plants, with the most substantial enhancement observed at 0.5 mM VA  
254 under water-deficient conditions (Table 1 and Fig. 3B).

255 Drought stress led to <sup>13</sup>a significant increase in the activities of superoxide dismutase (SOD),  
256 peroxidase (POD), and catalase (CAT) ( $P \leq 0.01$ ; 0.05; 0.001, respectively) <sup>90</sup>enzymes in the leaves  
257 <sup>90</sup>of the pea plants (Table 1). The priming of pea seeds with VA had a significant ( $P \leq 0.001$ ; 0.001;  
258 0.05) stimulating influence in accelerating the activities of SOD, POD and CAT, particularly under  
259 drought stress conditions (Table 1; Fig. 3CDE). Under both water regimes, 2.0, 3.0 and 4.0 mM  
260 VA markedly boosted the functionality of all antioxidant enzymes.

#### 261 **4 Discussion**

262 Water scarcity is recognized as a primary factor causing a major decline in crop bio-mass  
263 and yields all over the world, particularly in water-scarce and semi-arid areas (Akram et al., 2023).  
264 It is widely known that water deficit conditions profoundly affect the <sup>58</sup>growth and development of  
265 almost all plant <sup>58</sup>species (Shafiq et al., 2014; Ahluwalia et al., 2021; Seleiman et al., 2021) because  
266 adequate water availability is essential at each phase of a plant's life cycle. This challenging  
267 condition may lead to a <sup>19</sup>reduction of more than 50% in the average yield of major <sup>34</sup>crops (Wang et  
268 <sup>19</sup>al., 2003; Ashraf et al., 2011; Lamaoui et al., 2018). However, there is <sup>34</sup>substantial evidence that  
269 plants can adjust themselves by altering physiological, biochemical, and anatomical features in

270 response to both internal and external climate related factors including drought stress (Naz et al.,  
271 2023). Through the adaptive mechanisms including osmoprotection, osmotic adjustment, ionic  
272 compartmentalization, upregulation of antioxidants and accumulation of stress hormones, plants  
273 can endure and thrive well in challenging environmental conditions (Shafiq et al., 2015; Akram et  
274 al., 2016; Naz et al., 2023). Moreover, a multitude of strategies are in vogue to alter plants that  
275 could flourish well under harsh environmental indications. Of those, seed priming is considered a  
276 shotgun and efficient technique for promoting plant growth under stressful regimes (Ashraf and  
277 Foolad, 2005; Akram et al., 2020). However, for seed priming, a variety of organic and inorganic  
278 chemicals are currently under use (Akram et al., 2020; Kong et al., 2023).

279 In the current investigation, water stress significantly decreased both the fresh and dry  
280 weights of shoots and roots in the pea plants, whereas seed priming with different levels of vanillic  
281 acid triggered an important improvement in the fresh and dry weights of the pea plants (Table 1;  
282 Fig. 1) under varying water regimes. Overall, 4.0 mM and 6.0 mM levels of VA were more  
283 effective in enhancing the fresh and dry weights of the pea plants. Since no relevant literature is  
284 available to exhibit the role of the exogenous application of VA (a phenolic compound) to plants,  
285 the results recorded here could be treated as the first study in this regard. Nonetheless, Moran-  
286 Palacio et al. (2014) observed a positive relationship between total phenolic content and  
287 antioxidant properties in *Rhizophora mangle* and *Krameria erecta* plants. Furthermore, vanillic  
288 acid and p-hydroxy benzoic acid were identified as growth-promoting substances that mitigated  
289 leaf contraction and senescence in sorghum exposed to temperature stress conditions (Ahmad et  
290 al., 2016). Consequently, it is plausible to suggest that the foliar treatment of VA may play a vital  
291 role in triggering the production of osmoprotectants, to enhance drought resistance and improve  
292 the survival capacity of plants under water-limited conditions as found in rice (Ahmad et al., 2016).

293 The relationship between reduced water content and the adaptability of cell membranes to  
294 withstand various environmental signals, including those of drought stress has already been widely  
295 reported (Liu et al., 2002; Ahmad et al., 2016; Yang et al., 2021). Moreover, under water-deficit  
296 conditions, the sustainability and permeability of cell membranes tend to decrease plant growth  
297 (Blokhina et al., 2003). When assessing the physiological implications of cellular water scarcity,  
298 LRWC is considered a potential criterion for evaluating the water status of plants. However,  
299 consistent with earlier reported studies, the findings of the current study demonstrated that LRWC  
300 was significantly affected in the drought-stressed pea plants. However, VA exogenous application

301 had a positive effect on LRWC, which is consistent with the findings of Hura et al. (2012). They  
302 reported a notable increase in phenolics bound to the cell wall, which were associated with  
303 enhanced water retention within the plant. This led to a delay in leaf desiccation and the  
304 development of leaf undulations. Additionally, these phenolic compounds, including derivatives  
305 of hydroxycinnamic acids (such as ferulic acid and p-coumaric acid) and flavonoids (such as  
306 kaempferol and quercetin), were localized in the cell walls and vacuoles of the epidermis. This  
307 localization potentially functions as a photoprotective mechanism for the photosynthetic apparatus,  
308 providing defense against the potential damage to leaf cell structures caused by UV radiation (Hura  
309 et al., 2012; Nichols et al., 2015).

310 Under water deficit conditions, chlorophyll pigments play a crucial role in energy dissipation  
311 and light absorption during photosynthesis (Akram et al., 2018). In the existing study, scarcity of  
312 water led to a decline in photosynthetic pigments in the pea plants. This reduction in pigments  
313 under water shortage is a commonly observed reaction across various crops, such as mung bean  
314 (Batra et al., 2014), potato (Arabshahi and Mobasser, 2017), chickpea (Mafakheri et al., 2010),  
315 carrot (Razzaq et al., 2017), and canola (Akram et al., 2018), suggesting a shared adaptive  
316 mechanism of plants to drought conditions. The decrease in chlorophyll levels can be attributed to  
317 excessive production of ROS, disruptions in nutrient balance, and disturbances in enzyme  
318 activities caused by cellular or plant-level water deficiency. In accordance with these findings, the  
319 current study exhibited a reduction in chlorophyll *a* content under water deficit situations.  
320 However, the use of VA pointedly mitigated the harmful effects of drought on the pea plants.  
321 Likewise, Xuan and Khang (2018), reported that foliar application of low concentrations of vanillic  
322 acid increased the chlorophyll contents in rice plants.

323 Two essential osmolytes, glycinebetaine (GB) and proline, are known to accumulate in  
324 numerous crop species under stressful conditions and they play a critical part in osmotic  
325 modification (Raza et al., 2016). Raza et al. (2014) conveyed that a high accumulation of GB  
326 enhanced plant tolerance to various abiotic stresses, together with water shortage. The  
327 accumulation of GB at a high level enhanced the drought resistance of plants by promoting the  
328 functionality of antioxidant enzymes (Ma et al., 2014) and maintaining turgor pressure (Ashraf  
329 and Foolad, 2007). In the deficient supply of water, the decrease in leaf water potential triggers an  
330 accelerated synthesis of GB, which helps maintain the osmotic potential of leaves (Ashraf and  
331 Foolad, 2007). Moreover, proline is also known for its role in protecting plants against ROS and

332 regulating osmoregulation (Aranjuelo et al., 2010; Yaqoob et al., 2019). This study revealed a  
333 noteworthy elevation in the concentration of both proline and GB, particularly under the water  
334 stress level of 60% F.C. Studies on radish plants (Akram et al., 2016) and rice by Galahitigama  
335 and Wathugala (2016) demonstrated that the enhanced accumulation of proline and GB under  
336 drought stress contributed to increased stress tolerance. Moreover, in the current study, the  
337 exogenous spray of vanillic acid augmented the concentration of proline and GB contents in the  
338 pea plants subjected to water stress as well as normal watering. However, VA-induced  
339 accumulation in GB or proline could not be linked with any earlier investigation, as no report is  
340 available in the literature on this aspect.

341 The plant's defense against oxidative stress includes both enzymatic and non-enzymatic  
342 antioxidants, which shield plant cells from damage caused by drought stress. Some investigations  
343 have demonstrated that augmentation of the antioxidative defense system could enhance drought  
344 tolerance across various plant species, e.g., radish (Shafiq et al., 2015), and rice (Nounjan et al.,  
345 2012). Among non-enzymatic compounds, ascorbic acid is widely known for its ability to protect  
346 plants against various abiotic stresses by effectively rummaging oxy-gen-free radicals (Shafiq et  
347 al., 2014). Ejaz et al. (2012) stated that the cellular level of AsA is associated with the stimulation  
348 of plants' protective system. Ascorbic acid plays a crucial role in plant growth and is implicated in  
349 various physiological processes, e.g., division of cells, cell expansion, and several others (De Gara,  
350 2004). In this study, we observed a rise in ascorbic acid content in the pea plants subjected to  
351 drought stress, which is consistent with earlier findings in maize (Dolatabadian et al., 2010)  
352 wherein a significant elevation in AsA, particularly reported under high drought intensity.  
353 Furthermore, in our study, the use of VA enhanced the accumulation of ascorbic acid in water-  
354 deficit environments. Under drought-induced oxidative stress, phenolic compounds accumulate  
355 and protect fatty acids, as previously reported by Frary et al. (2010) and Amri et al. (2017). In the  
356 course of our investigation, we noted a rise in total phenolic content in the pea plants under water  
357 deficit conditions, which aligns with the findings recorded in maize (Moharramnejad et al., 2015),  
358 canola (Dawood and Sadak, 2014), and quinoa (Aziz et al., 2018) under drought conditions.

359 Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) are enzymatic  
360 antioxidants that perform a crucial role in neutralizing reactive oxidants, thereby en-hancing stress  
361 tolerance in crops against drought. Previous studies by Ashraf (2009) and Akram et al. (2018) have  
362 reported the involvement of these enzymes in the mechanism of drought tolerance in dif-ferent

363 plants. It has been suggested that the ability of a species to tolerate drought stress can be linked to  
364 the enhanced activity of antioxidant enzymes, as demonstrated by Lima et al. (2002) and Yadav  
365 and Sharma (2016). In our current study, we observed higher activities of SOD, CAT, and POD in  
366 the drought-stressed pea plants, similar to the findings reported earlier in canola (Akram et al.,  
367 2018) and radish (Shafiq et al., 2015) cultivars that exhibited increased enzyme activities under  
368 stress conditions. The exogenous application of VA positively influenced the actions of  
369 superoxide dismutase, catalase, and peroxidase enzymes in drought-hit plants, similar to the  
370 stimulation of catalase and superoxide dismutase for nullifying the reactive oxygen species as  
371 observed in rice under flooding conditions (Xuan and Khang, 2018). Numerous scientists have  
372 described the role of phenolic compounds in increasing the activities of superoxide dismutase and  
373 catalase in water-deficit plants for ROS detoxification (Abu El-Soud et al., 2013; Singh et al.,  
374 2019).

## 375 5 Conclusions

376 Seed priming with vanillic acid enhanced the growth and regulated physio-biochemical  
377 parameters of drought-stressed pea plants. It also improved these attributes in non-stressed control  
378 plants. These results suggested that vanillic acid treatment can be advantageous for promoting  
379 plant growth under both stressful and non-stressful conditions. So, the capability of vanillic acid  
380 to enhance stress tolerance in crop plants can provide valuable benefits to farmers facing water  
381 deficit stress conditions.

382

383

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602 processes and activity of antioxidant enzymes in plants of two cultivars of quinoa  
603 (*Chenopodium quinoa* Willd. *Plants* 8, 58.

604 **Table 1** Mean squares from ANOVA of data for different morphological and phys-io-biochemical  
 605 characteristics of pea (*Pisum sativum* L.) plants raised from seeds treated with varying levels of  
 606 vanillic acid and subjected to water-deficit conditions.

<b>Source of Variation</b>	<b>df</b>	<b>Shoot fresh weight</b>	<b>Shoot dry weight</b>	<b>Root fresh weight</b>	<b>Root dry weight</b>
Drought stress (D)	1	5.286***	0.099***	1.676**	0.063***
Vanillic acid (VA)	7	0.876***	0.057***	0.464**	0.019***
D x VA	7	0.198ns	0.006*	0.455**	0.002ns
		<b>Shoot length</b>	<b>Root length</b>	<b>Leaf area per plant</b>	<b>LRWC</b>
Drought stress (D)	1	568.5***	497.2***	4.557**	1750***
Vanillic acid (VA)	7	105.4***	95.14***	2.719***	366.3***
D x VA	7	13.22ns	20.42***	0.042ns	43.63ns
		<b>RMP</b>	<b>Chlorophyll a</b>	<b>Chlorophyll b</b>	<b>Total chlorophyll</b>
Drought stress (D)	1	298.5***	0.261*	0.038ns	0.262*
Vanillic acid (VA)	7	177.7***	0.114*	0.138***	0.114*
D x VA	7	6.659ns	0.021ns	0.002ns	0.021ns
		<b>Chlorophyll a/b ratio</b>	<b>Proline</b>	<b>Glycinebetaine</b>	<b>Ascorbic acid</b>
Drought stress (D)	1	0.792ns	0.799**	159.4***	15.36*
Vanillic acid (VA)	7	2.053ns	2.604***	107.8***	16.39***
D x VA	7	0.160ns	0.096ns	3.242ns	1.233ns
		<b>Total phenolics</b>	<b>SOD</b>	<b>POD</b>	<b>CAT</b>
Drought stress (D)	1	160.1***	1.693**	0.162*	0.042***
Vanillic acid (VA)	7	26.93***	1.815***	0.115***	0.006*
D x VA	7	2.477ns	0.042ns	0.005ns	0.001ns

607 \*, \*\* and \*\*\*= significant at 0.05, 0.01 and 0.001 levels, respectively; ns= non-significant.

# JKSUS Plagiarism Report

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