

1 **Word count:5546**

2 **Root interactions and plant growth in a tomato/potato onion intercropping system under**
3 **different phosphorus levels**

4

5 **Running title: root distributions in a mixed system**

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18 **KEYWORDS:** intercropping system; plant growth; root distribution; root tendency; P levels

19

20 **SUMMARY STATEMENT**

21 Our study can help more people clearly know the relationship between the root interactions and
22 plant growth in mixed cultures.

23

24 **ABSTRACT**

25 Intercropping systems have been implemented in many parts of the world due to their beneficial
26 effects on yield and biomass. In intercropping systems, changes in plant growth are usually
27 related to variations in root distribution and phosphorus (P) levels, however, root distributions
28 and root tendencies are difficult to study, as root systems grow beneath the soil surface.
29 Therefore, we have a relatively poor understanding of the relationship between plant root
30 interactions and plant growth in intercropping systems. In this study, a custom apparatus
31 consisting of a transparent manual root box was used to observe intact root systems in situ. We
32 investigated how root distribution and root tendency changed in a tomato/potato onion
33 intercropping system under two P treatments, and how tomato plant growth was influenced. The
34 results showed that the shoot and total biomass for the tomato plants were increased by
35 intercropping with potato onion plants under both tested P levels, the root distribution of
36 intercropped tomato plants was deeper than that of monocultured plants, and the tomato roots
37 tended to grow away from the potato onion roots. Our research reveals that a deeper and more
38 evasive root distribution is more conducive to the plant growth of the intercropped tomato.
39

40 INTRODUCTION

41 Roots are extremely important plant organs that mediate nutrient and water absorption
42 (Barber, 1995; Fang et al., 2011). Over the long evolutionary history of plants, different species
43 have developed varying degrees of root plasticity. Within the complex living environment, root
44 plasticity can be influenced by many factors (Karban, 2008), such as nutrient heterogeneity
45 (Fang et al., 2009; Hodge, 2004; Liao et al., 2001, 2004) and the presence of neighboring roots
46 (de Kroon et al., 2003; Dudley and File, 2007; Falik et al., 2003; Karban and Shiojiri, 2009;
47 Maina et al., 2002).

48 In recent years, the topic of root recognition has attracted the attention of a growing number
49 of scholars. The presence of a neighboring plant that represents a resource competitor can trigger
50 an increase in root biomass allocation (Falik et al., 2003; Gersani et al., 2001; Maina et al., 2002;
51 O'Brien et al., 2005; Padilla et al., 2013), whereas some plants can recognize other individuals of
52 their own species and limit root proliferation (Biedrzycki and Bais, 2010; Biedrzycki et al., 2010;
53 Dudley and File, 2007). For example, when planted with non-kin species instead of siblings, the
54 Great Lakes Sea Rocket (*Cakile edentula*) accumulates more biomass in its fine roots (Dudley
55 and File, 2007). In addition, *Impatiens pallida* plants are capable of kin recognition only when
56 the roots of another plant are present (Murphy and Dudley, 2009). Therefore, when analyzing
57 intercropping systems, which are practiced in many parts of the world, including tropical,
58 subtropical and temperate regions (Francis, 1986; Vandermeer, 1989), the process of root
59 recognition should be considered.

60 Intercropping can confer considerable yield and biomass advantages in certain situations
61 (Awal et al., 2006; Tsubo and Walker, 2002; Zhang et al., 2007). With respect to intercropping
62 systems, most studies have addressed interspecific facilitation, in which different plant species
63 benefit one another when two species are grown together. Interspecific facilitation can benefit
64 plant growth and nutrient absorption after intercropping with other species in agro-ecosystems
65 (Ae et al., 1990; Cu et al., 2005; Gardner and Boundy, 1983; He et al., 2013; Horst and
66 Waschki, 1987; Li et al., 2001, 2003, 2007, 2010). However, as root systems are hidden below
67 ground, it can be difficult to observe and quantify root growth in situ, explaining why there are
68 relatively few studies concerning root interactions in these important agricultural systems. Li et
69 al. (2006) and Gao et al. (2010) confirmed that greater lateral root deployment and compatibility
70 of spatial root distribution in intercropping species contribute to higher yields and plant growth.

71 Xia et al. (2013) suggested that total root length growth and the spatial distribution of roots are
72 sensitive to phosphorus (P) application in cropping systems (Xia et al., 2013). Phosphate ions in
73 soil usually become unavailable by reacting with soil cations to form either soluble complexes or
74 insoluble precipitates (Cu et al., 2005), or they adsorb to the surfaces of various positively
75 charged soil particles (Hinsinger et al., 2003). Based on these investigations, we can conclude
76 that plant root behavior is much more complex than previously thought.

77 Tomato (*Solanum lycopersicum* L.) is a widely cultivated vegetable around the world,
78 although continuous monocropping of tomato plants and excessive fertilizer application have
79 resulted in soil acidification and salinization in many locations, decreasing tomato yields and
80 fruit quality (Liu et al., 2014). The potato onion (*Allium cepa* L. var. *aggregatum* G. Don) is an
81 onion variety that is widely cultivated in northeastern China and is a good companion plant for
82 tomato. In many previous studies, intercropping of tomatoes and potato onions has been shown
83 to increase tomato quality, alleviate tomato *Verticillium* wilt and improve soil quality by altering
84 soil enzyme activities and microbial communities (Fu et al., 2015, 2016; Liu et al., 2014;
85 Tringovska et al., 2015; Wu et al., 2013, 2016). However, we know little about how the roots of
86 these species interact when tomato plants are intercropped with potato onion plants, and in
87 particular, it is unknown whether distinct root architectures appear when the intercropping plants
88 respond to different P levels. In the present study, we used a custom apparatus consisting of a
89 transparent manual root box to observe the root system in situ in a non-destructive manner. We
90 tested how root distributions and root tendencies changed in tomato/potato onion intercropping
91 systems with no P added or 120 mg·kg⁻¹ P added. We measured changes in tomato plant growth
92 and analyzed how plant interactions affected cropping patterns and P levels. We hypothesized
93 that the spatial root distribution of the tomato and potato onion plants would be compatible yet
94 distinct at both P levels, contributing to increased plant growth.

95

96 **RESULTS**

97 ***Influence of cropping patterns and P levels on tomato plant growth***

98 The cropping pattern and P level treatments both affected shoot biomass and total plant
99 biomass in the tomato plants, and we observed a significant P level × cropping pattern interaction
100 for shoot biomass and plant total biomass (P>0.05; Table 1). Compared with the biomass of
101 monoculture tomato plants, the shoot biomass and total plant biomass of the tomato plants

102 increased significantly when intercropped with potato onion for both no P added and 120 mg·kg⁻¹
103 P added treatments. For tomato root biomass, we did not detect any statistically significant
104 interactions between the cropping pattern and P level treatments. However, tomato root biomass
105 was clearly influenced by the cropping pattern (P>0.05; Table 1), although it was not
106 significantly influenced by the P level.

107

108 ***Root length density distribution***

109 Influenced by both neighboring plants and the P application rate, RLD was unevenly
110 distributed in the mixed cultures (Fig. 1A, B, G, and H) but evenly distributed in the tomato
111 monoculture (Fig. 1E and K) and the potato onion monoculture (Fig. 1C and L). When tomato
112 plants were intercropped with potato onion plants, the RLD area of tomato (Fig. 1A and G) was
113 much deeper than in the tomato monoculture (Fig. 1E and K). In the mixed cultures, the roots of
114 the tomato plants (Fig. 1A and G) always avoided contact with the roots of the potato onions,
115 whereas the roots of the potato onion (Fig. 1B and H) spread laterally under the neighboring
116 tomato plants under both P application treatments.

117 The root tendency of plants neighboring the same species was more strongly influenced by P
118 level. In the no P added treatment, the RLD areas of the potato onion and tomato were not
119 intermingled when neighboring the same plant species (Fig. 1D and F). By contrast, for the 120
120 mg·kg⁻¹ P added treatment, the RLD areas of potato onion plants neighboring the same species
121 (Fig. 1J) were markedly intermingled, whereas the RLD areas of tomato plants neighboring the
122 same species (Fig. 1L) were clearly separated.

123

124 ***Root weight density distribution***

125 The root weight density distribution is shown in Fig. 2. Under the no P added and 120
126 mg·kg⁻¹ P added treatments, the 0.2 g kg⁻¹ soil root weight density (RWD) contour of the
127 intercropped tomato plants (Fig. 2A and G) occupied a deeper soil layer than that of the tomato
128 monoculture, and under the no P added treatment, the RWD of the tomato plants was higher than
129 in the monoculture. The 2 g/kg soil RWD contour distribution of potato onion plants (Fig. 2B
130 and H) was distributed in a narrower area than in the monoculture (Fig. 2C and I), and the RWD
131 of the intercropped potato onion plants (Fig. 2B and H) was higher than for the monocultures

132 (Fig. 2C and I). The directions of RWD in both intercropping systems and monocultures were
133 the same as for RLD.

134 In general, the root distributions of the tomato plants became deeper when intercropped with
135 potato onion plants than those under tomato monoculture, and under the no P added conditions,
136 the RWD of the tomato plants became higher than in monoculture. For the two P levels, the root
137 tendencies of the two crops were significantly different, with the tomato roots avoiding contact
138 with potato onion roots and the potato onion roots clearly extending towards the tomato roots.
139 When the tomato plants were next to the same species, their roots were crossed under the no P
140 added treatment, whereas they avoided crossing under the 120 mg·kg⁻¹ P added treatment. The
141 root distribution of the potato onion plants in the intercropping system became narrower than in
142 monoculture. Additionally, the root tendency of the potato onion plants neighboring the same
143 species was opposite to that of the tomato roots: under the no P added treatment, the root areas of
144 the potato onion plants neighboring the same species were separated, whereas under the 120
145 mg·kg⁻¹ P added treatment, the roots were significantly crossed.

146

147 ***Root tendency in root boxes***

148 Image data were obtained on the 12th day after transplantation (sampling time was tested in
149 our previous experiment to ensure that the roots of two plants in one box remained uncrossed).
150 Fig. 3 shows how the root architecture was affected by neighboring plants under the no P added
151 treatment in the root box. In P0MT1 (one tomato plant in monoculture) and P0MO1 (one potato
152 onion plant in monoculture), the roots of tomato plants and potato onion plants were distributed
153 evenly. In the other combinations, the roots were unevenly distributed to a significant extent. In
154 P0I (the tomato/potato onion intercropping system), the tomato roots avoided the potato onion
155 roots significantly, whereas the potato onion roots spread laterally under the tomato row. In
156 P0MT2 (two tomato plants in monoculture), the roots of the two tomato plants were clearly
157 crossed, whereas the potato onion roots avoided other potato onion roots in P0MO2 (two potato
158 onion plants in monoculture).

159 For the 120 mg·kg⁻¹ P added treatments (Fig. 4), the root distributions for the different
160 treatment combinations were different from those under the no P added treatments. In PMT1
161 (one tomato plant in monoculture) and PMO1 (one potato onion plant in monoculture), the
162 tomato and potato onion roots were evenly distributed, which is associated with P deficiency. In

163 PI (the tomato/potato onion intercropping system), the tomato roots also avoided the potato
164 onion roots, whereas the potato onion roots spread in a nearly uniform distribution. In PMT2
165 (two tomato plants in monoculture) and PMO2 (two potato onion plants in monoculture), the
166 root tendencies of the tomato plants and potato onion plants exhibited opposite trends to those
167 under the no P added treatment; in PMT2, the tomato roots avoided intermingling, whereas the
168 potato onion roots showed no significant trend.

169

170 ***Root percentage distribution in root boxes***

171 Consistent with Figs. 3 and 4, the distribution of the root percentage is shown in Fig. 5. In
172 the tomato/potato onion mixed culture, the tomato root length percentage of P0IT in space 2 was
173 significantly higher than in PIT, and the tomato root length percentage of P0IT in the 6–9-cm
174 spaces was lower than in PIT, indicating that tomato roots avoided potato onion roots more
175 clearly than under the no P added treatment. In the P0MT2 treatment, the root length percentage
176 in the middle area was higher than on the two sides, with horizontal distance 16-cm showing the
177 highest percentage. In PMT2, the root length percentage was higher in horizontal distance 2–6-
178 cm and 24–28-cm than in 12–18-cm. Specifically, when tomato plants were intercropped with
179 the same species, their root growth trend was related to the P level, with the roots crossing under
180 the no P added treatment, whereas they clearly avoided one another under the 120 mg·kg⁻¹ P
181 added treatment.

182 Fig. 5 shows that the root length percentage of potato onion plants in P0IO was higher on
183 the left than on the right, and the root length percentage in horizontal distance 10–18-cm was
184 higher than in PIO. In PIO, the potato onion roots were distributed evenly on both sides,
185 revealing that the potato onion roots tended to extend towards the tomato roots when the species
186 were intercropped under the no P added treatment, although this trend was not significant under
187 the 120 mg·kg⁻¹ P added treatment. In P0MO2, the root length percentages on both sides (2–4-cm
188 and 26–28-cm) were higher than in the middle zone (12–18-cm), whereas in PMO2, there was no
189 obvious trend. In other words, when potato onion plants were intercropped with the same species
190 under no P added treatments, the roots avoided mixing, whereas there was a less obvious trend
191 under the 120 mg·kg⁻¹ P added treatments. All of these data are consistent with the results
192 presented in Figs. 3 and 4.

193

194 **DISCUSSION**

195 Enhancement of plant biomass under intercropping has been observed in many experiments
196 (Awal et al., 2006; Li et al., 1999, 2001; Tsubo and Walker, 2002; Xia et al., 2013; Zhang et al.,
197 2007). In our experiments, results for both P treatments showed that aboveground tomato
198 biomass and plant total biomass could both be increased significantly by intercropping with
199 potato onion plants. We detected statistically significant interactions between the cropping
200 patterns and P level treatments for both shoot biomass and total plant biomass of tomato in the
201 intercropping system. Regarding the root dry weights of the tomato plants, we did not detect any
202 statistically significant interactions between cropping pattern and P level treatments, although
203 this metric was clearly influenced by cropping pattern ($P>0.05$) and not significantly influenced
204 by the P level. In previous studies, few experiments have addressed the effects of the interaction
205 between cropping patterns and P levels on plant growth in intercropping systems. However, Li et
206 al. (2008) and Wang et al. (2007) have shown that root biomass can be differentially affected in
207 different intercropping system combinations, increasing in some contexts while remaining the
208 same as in monocultures in others, suggesting that in mixed cultures, root biomass can be
209 influenced by the species type of adjacent plants. In addition, Li et al. (2010) showed that the
210 root biomasses of different crops can vary under different P levels. Thus, the root biomass in
211 intercropping systems can be affected by many factors. In our experiment, the root biomass of
212 tomato plants was significantly influenced by intercropping tomato plants with potato onion
213 plants.

214 Root interactions have been studied in many intercropping systems, and the spatial
215 distribution of roots and their density in the soil has been shown to determine the ability of a crop
216 to acquire the necessary nutrients and water to sustain growth. In this study, the roots of tomato
217 plants and potato onion plants both showed an extended root distribution, and the RLD and
218 RWD of the tomato plants both became deeper than in tomato monoculture, consistent with
219 previous literature (Adiku et al., 2001; Gao et al., 2010; Li et al., 2006). Some studies have found
220 that the root distribution can become unbalanced and that roots can extend horizontally to greater
221 distances in an intercropping system (Zhang and Huang, 2003), with overyielding of species
222 resulting from the greater lateral deployment of roots and increased RLD. The roots of
223 intercropped plants can extend into the root area of other plants and sometimes penetrate deeper
224 than in monoculture (Adiku et al., 2001; Li et al., 2006), and the compatibility of the spatial root

225 distributions of the intercropped species contributes to interspecific facilitation. In our
226 experiment, the extension of the root distribution and the deeper root space of tomato plants may
227 have contributed to increased plant biomass. However, the root tendencies of the two crops
228 observed here were not the same as in previous studies. In previous studies by Adiku et al.
229 (2001) and Li et al. (2006), the roots of two crops were found to extend into each other's root
230 areas. Thus, we believe that the root tendency of one plant in an intercropping system may be
231 influenced by both plant species, and whether the roots of the two crops are mixed or separate,
232 these different tendencies are beneficial for plant nutrient absorption. These tendencies generally
233 involve an extended root distribution, and greater root length can help plants absorb nutrients and
234 increase biomass.

235 In previously studies, the results of competition have always been connected to resources
236 and plant species. Some authors believe that the results of competition can be variable in
237 different environments, with intraspecific competition being dominant under some conditions
238 (Sheley and Larry, 1994; Velagala et al., 1997; Wassmuth et al., 2009), whereas interspecific
239 competition is stronger under others (Blank, 2010; Vasquez et al., 2008; Young and Mangold,
240 2008). Ge et al. (2000) demonstrated that low inter-root competition is a more efficient way for
241 adjacent plants to decrease root overlap, and Zhang et al. (2002) showed that when root weight is
242 at its maximum and roots do not overlap in a wheat/faba bean intercropping stage, then
243 competition between the two crops for water and nutrients can be reduced, resulting in higher
244 yields for both species. In our intercropping system, the root action of tomato plants was
245 consistent with that observed for this previous study, and under a nutrient-deficient conditions,
246 the roots opted to decrease their overlap and decrease their competition with potato onion plants.

247 In our analysis of root tendency, when the tomato and potato onion plants were planted with
248 their same species, the reaction of the roots was more closely related to the P level. The roots of
249 the potato onion plants were clearly separated from those of their same-species neighbors under
250 no P added treatment, which appeared to aid in avoiding competition and improving survival of
251 the species, whereas no obvious root tendency was observed in the absence of P stress. However,
252 the tomato roots intermingled with one another under the no P added treatment, possibly
253 allowing them to compete for more resources, whereas the roots clearly avoided intermingling
254 under the 120 mg·kg⁻¹ P added treatment, possibly to avoid competing for resources. Cheplick
255 and Kane (2004) reported when two kin plants are planted together, their roots can avoid one

256 another or engage in spatial segregation to avoid competing for resources, whereas when non-kin
257 plants are planted together, the roots usually overlap, allowing for more competition. In these
258 experiments, the root behavior of the potato onions was consistent with previous results, perhaps
259 because in this variety was not subjected to artificial transformation. Or in other words, for the
260 potato onion, the results regarding root recognition appeared biased towards protecting the
261 species itself, thus preventing competition among roots under no P added treatment. However,
262 the responses of the tomatoes were different from those of Cheplick and Kan. Generally, studies
263 on kin recognition have been conducted on wild plants, whereas few such studies have been
264 performed on crop species (Dudley and File, 2007; Murphy and Dudley, 2009). Wild plants
265 usually grow under natural conditions in which resources are limited; however, in some long-
266 term cultivated species grown under resource-rich conditions (Wenke, 1980), the ability of roots
267 to recognize those of their kin have gradually decreased, and root recognition can be affected by
268 plant species and genotype in a significant manner (Fang et al., 2011). Therefore, considering
269 that the tomato seeds we selected have been subjected to long-term cultivation, we speculate that
270 the root recognition may have been weakened in these plants. Or in other words, when planted
271 with their siblings under nutrient-deficient conditions, these plants no longer know to protect
272 their kin. The results of our study clearly show that tomato and potato onion roots can respond to
273 nutrients and adjacent plants, consistent with the viewpoint of Cahill et al. (2010), although
274 determining which factors in an intercropping system are most important for controlling root
275 behavior requires further research.

276

277 **MATERIALS AND METHODS**

278 ***Plant materials and cultivation conditions***

279 The tomato (*Solanum lycopersicum L.*) variety “Dongnong708” was provided by the
280 Tomato Breeding Center of Northeast Agricultural University (Harbin, China). The potato onion
281 (*Allium cepa var. agrogatum Don.*) variety Suihua, a native variety with potential allelopathy
282 (Liu et al., 2013), was provided by the Laboratory of Vegetable Physiological Ecology (Harbin,
283 China). Tomato seeds were treated with hot (55°C) water and germinated in Petri dishes with
284 wet gauze in the dark at 28°C. Seedlings with two cotyledons were planted in plastic pots (8×8
285 cm) containing 100 g soil after emergence, and seedlings with four leaves were then used for the
286 different experiments. All of the seedlings were cultivated in a phytotron located in the

287 Experimental Center at Northeast Agricultural University, Harbin, China (45°41'N, 126°37'E),
288 from July 2014 to October 2015, and the phytotron was maintained under the following
289 conditions: 14/10 h light/dark cycle, 28/18°C day/night temperature and 70% air relative
290 humidity. The potato onion plants were stored at 4°C before planting. All of the experiments
291 were performed in the Laboratory of Vegetable Physiological Ecology (Harbin, China).

292

293 ***Experiment 1: Tomato/potato onion mixture in pots***

294 The primary pot treatments consisted of no additional added P and 120 mg·kg⁻¹ P added.
295 These P concentrations were based on previous experimentation showing that soil with no
296 additional P is insufficient for tomato growth and that soil with 120 mg·kg⁻¹ P added is sufficient.
297 The sub-pot treatments addressed tomato/potato onion intercropping and tomato monoculture in
298 plastic pots (28 cm diameter, 20 cm height) containing 3 kg soil. At the time of tomato
299 transplantation, the potato onion plants were planted, and the tomato:potato onion ratio was 1:3
300 in the intercropping treatment. The experimental design was a randomized complete block
301 design with three replicates. Four treatments were performed in each block, and 4 pots were
302 included in each treatment. In all, there were 16 pots per block and with 3 blocks total, yielding
303 48 pots in all. Each pot was watered with tap water every 3 days to maintain the soil water
304 content at approximately 60% of the water-holding capacity, and the plants were grown in the
305 phytotron as described above.

306 Sandy loam soil from the 30-50 cm layer under the ground surface was collected from an
307 open field at Northeast Agricultural University (Harbin, China). The soil contained 17.4 g·kg⁻¹
308 organic matter, 40.6 mg·kg⁻¹ available N (nitrate and ammonium), 11.4 mg·kg⁻¹ Olsen P and
309 100.9 mg·kg⁻¹ available K, and it exhibited an electrolytic conductivity (1:5, w:v) of 153.5
310 mS·cm⁻¹ and a pH (1:5, w:v) of 6.98. Previous experiments have shown that even when the total
311 P and available P levels are relatively high, soil can still be considered P-deficient for plants if
312 plant growth can be improved by P addition (Holloway et al., 2001; Li et al., 2005; Wang et al.,
313 2007). In a previous experiment, we confirmed that the base soil P content was insufficient for
314 tomato plants (data not shown).

315 P was added as KH₂PO₄ at 120 ppm for the 120 mg·kg⁻¹ P added treatment, and fertilization
316 with 120 ppm N (in the form of CO(NH₂)₂) and 120 ppm K (in the form of K₂SO₄) was
317 performed for both the no P added and 120 mg·kg⁻¹ P added treatments; then K₂SO₄ was used to

318 balance the K rate for the two P level treatments. Plants were harvested on the 20th day after
319 transplantation, thoroughly washed with distilled water and separated into roots and shoots. The
320 shoots and roots were killed by heating at 105°C for 30 min and then dried at 60°C for 72 h.

321

322 ***Experiment 2: Tomato/potato onion mixture in foam boxes***

323 The same soil and fertilizer management techniques described above were used in this
324 experiment. To provide sufficient space for the plant roots and to reduce harm to the root system
325 when sampling, we employed large foam boxes with an internal volume of 36×25×22 cm as
326 culture pots. Each foam box was filled with 20 kg soil. The experimental design was a
327 randomized complete block design with two replicates, and ten treatments were used in this
328 experiment. The primary pot treatments were no P or 120 mg·kg⁻¹ P added, and the sub-pot
329 treatment consisted of five intercropping combinations: 1) a tomato/potato onion intercropping
330 system, 2) one tomato plant in monoculture, 3) two tomato plants in monoculture, 4) one potato
331 onion in monoculture and 5) two potato onion plants in monoculture. The tomato to potato onion
332 ratio in the intercropping treatment was 1:3. When considering the nutrient balance per each box,
333 the three potato onion plants were viewed as equivalent to one plant.

334 The plants were sampled on the 20th day after transplantation, and root samples were
335 collected using the monolith method, as modified by Li et al. (2006) and Smit et al. (2013).
336 Briefly, the foam box was cut into vertical sections at 10-cm intervals along the wide side, the
337 soil surface was made as smooth as possible, and the roots were then fixed in each 6×4-cm area
338 with 5 cm nails. Finally, a 6×5×4 cm inner-diameter iron box was used to remove a 5 cm layer of
339 soil from the center of the foam box; the volume of each soil block was 120 cm³. There were 30
340 monoliths (5 in a vertical and 6 in a horizontal direction) in each soil profile, and 600 monoliths
341 were sampled in total. Each soil sample was placed in a numbered plastic bag.

342 All of the soil samples were poured onto a sieve (0.2 mm mesh, 30 cm diameter, 5 cm
343 height) and stirred until all of the roots could be freed of soil using very fine tweezers. The sieves
344 were suspended in a large water bath and shaken continuously, and the soil material remaining in
345 the sieves was removed manually. The tomato and potato onion roots were distinguished by
346 differences in color, smell and fibrous roots. For example, tomato roots are yellowish and hairy,
347 whereas potato onion roots have a smooth surface with white coloration and some degree of
348 transparency.

349

350 ***Experiment 3: Tomato/potato onion mixture for imaging the root tendency in the root boxes***

351 This experimental design was the same as experiment 2, and the treatments were assessed in
352 a transparent manual root box (a practical invention patent application has been filed for this
353 box). The root box consisted of transparent glass pieces and thirteen layers of nylon mesh with a
354 1 mm aperture, allowing us to image root architecture in situ without destroying the roots. Each
355 root box was filled with a 15 kg mixture consisting of one part sand and 3 parts vermiculite. For
356 the experiment, the plants were irrigated with modified Hoagland's nutrient solution, and the no
357 P added and 120 mg·kg⁻¹ P added treatments were created by adding P₂O₅ and KH₂PO₄ at 80 μM
358 and 320 μM concentrations, respectively. N and K were applied as CO(NH₂)₂ and K₂SO₄,
359 respectively, at 100 ppm, and K₂SO₄ was used to balance the K rate in both treatments. Other
360 nutrients were provided at the concentrations indicated by Fontes et al. (1986).

361 At the time of sampling, we removed the bottom of the root boxes and soaked them in water.
362 Half an hour later, when the sand and vermiculite were almost washed free, the root box was
363 gently removed and the culture medium was thoroughly rinsed from the root surfaces by
364 spraying. Two wooden sticks were run through the 13 networks from two sides, and the wire
365 frame was fixed with plastic grips. Two flashlights were used as a light source when taking
366 photographs. After imaging, the roots between every two grids were cut with a pair of scissors as
367 an individual sample, and the root length of each sample was determined using a root system
368 scanner, which we used to calculate the root percentage.

369

370 ***Statistical analysis***

371 Results regarding plant growth were analyzed using the SAS 8.0 software program (SAS
372 Institute Inc., Cary, USA), and the means of the different treatments were compared using
373 Tukey's test at the $p = 0.05$ level. The data are expressed as the means with standard errors. We
374 used general linear models to determine the significance of the primary effects (P level and
375 cropping pattern) and interactions (P level × cropping pattern) on tomato plant growth. Data
376 from the monoliths from the white foam box experiment represent the entire root population in
377 each soil profile. The results are presented as contour diagrams. Root length density (RLD)
378 contour diagrams were prepared using the Surfer v. 8.0 software program (Golden Software Inc.,
379 Golden, CO). Images from the root box experiment were obtained in panoramic view using an

380 Apple mobile phone. The root length percentage was determined using an image scanner
381 analyzer (LA - S2400).

382

383 **CONCLUSIONS**

384 Our study provides novel findings regarding plant growth and root interactions in an
385 intercropping system under differing P levels. Based on our results, we conclude that deeper and
386 more evasive root distributions in tomato plants can support greater tomato biomass in a
387 tomato/potato onion intercropping system.

388

389 **ACKNOWLEDGEMENTS**

390 We would like to thank Dr. Long Li (Department of Plant Nutrition, China Agricultural
391 University) for his valuable guidance in our experiment's design and result analysis.

392

393 **COMPETING INTERESTS**

394 The authors have no conflicts of interest to declare.

395

396

397 **FUNDING**

398 This research was financially supported by the National Natural Science Foundation of China
399 (Project No. 31672200) and National Staple Vegetable Industrial Technology Systems of China
400 (CARS-25).

401

402 **SYMBOLS AND ABBREVIATIONS**

403 Phosphorus-P

404

405 **REFERENCES**

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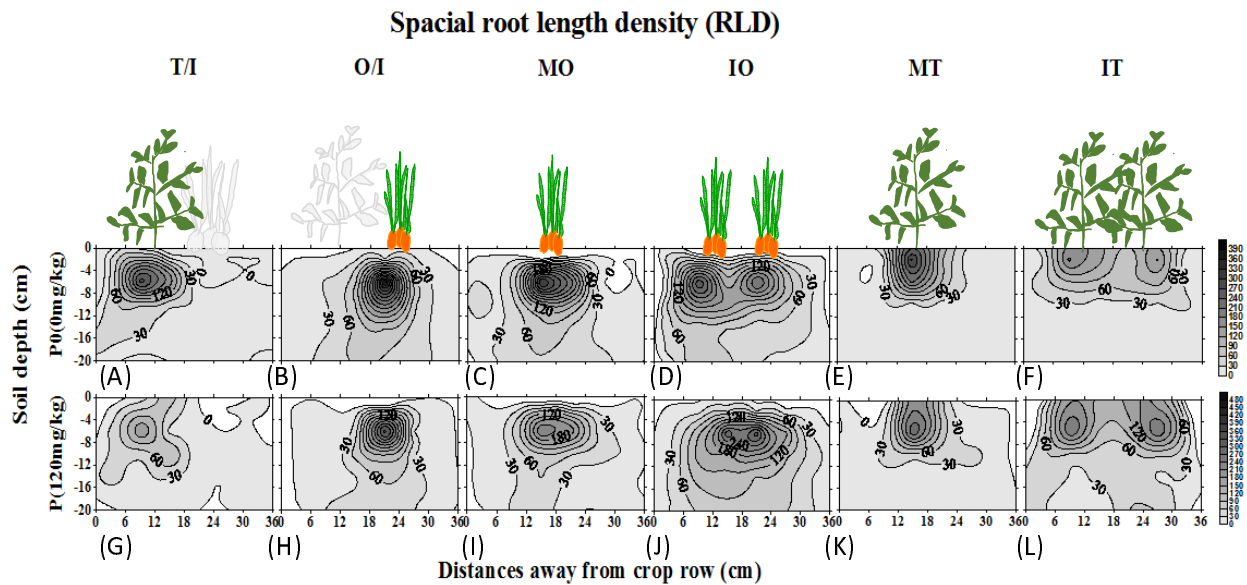
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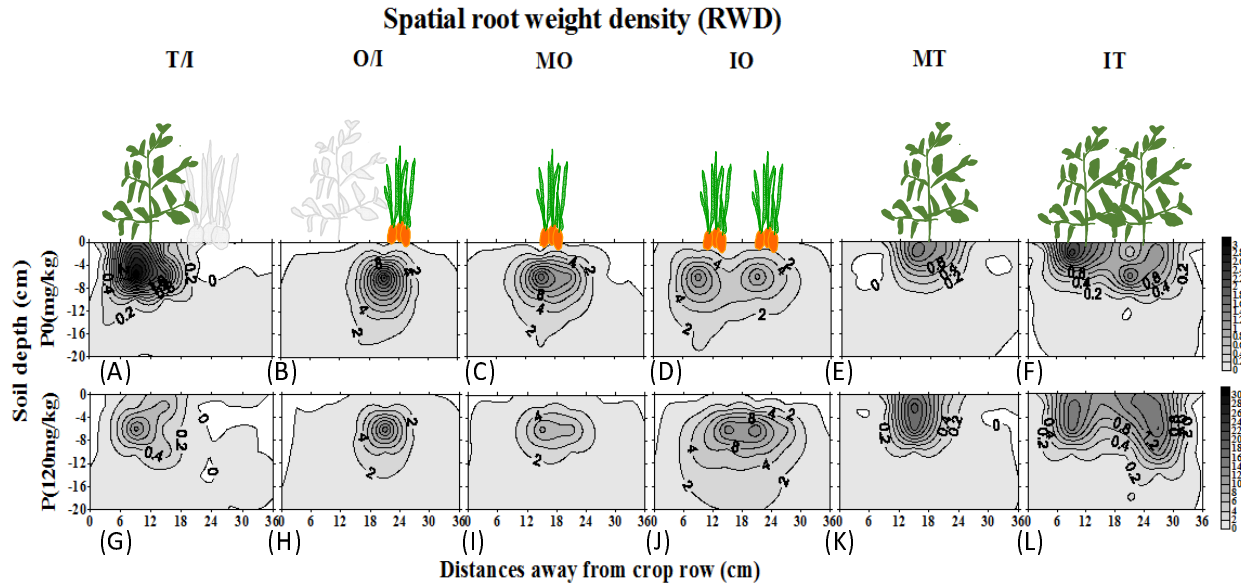
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569

570 **TABLE AND FIGURE LEGENDS**

571 **Fig. 1.** Spatial root length density (RLD) (cm per 120 cm³ soil volume) for various treatments
572 [(A, G) intercropped tomato, (B, H) intercropped potato onion, (C, I) one potato onion plant
573 monoculture, (D, J) two potato onion plants monoculture, (E, K) one tomato plant monoculture,
574 (F, L) two tomato plants monoculture] under no P added and 120 mg·kg⁻¹ P added treatments.
575 The contour lines are at intervals of 1 cm/12 cm³ soil volume.

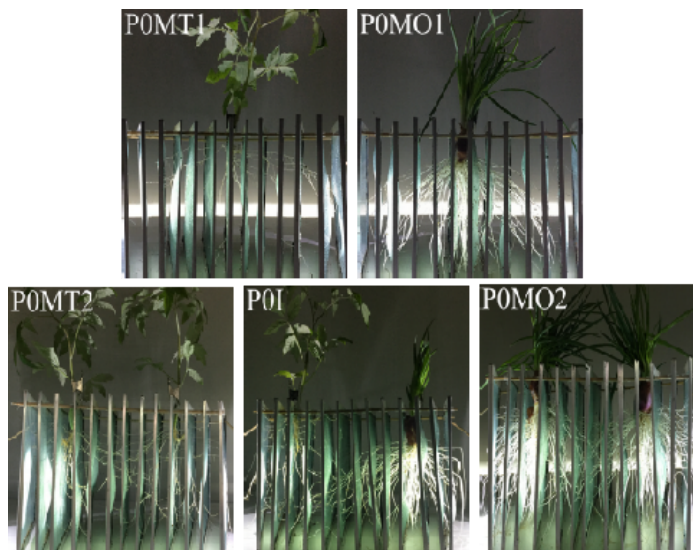


576 **Fig. 2.** Spatial RWD (g/kg) for various treatments [(A, G) intercropped tomato, (B, H)
577 intercropped potato onion, (C, I) one potato onion plant monoculture, (D, J) two potato onion
578 plants monoculture, (E, K) one tomato plant monoculture, (F, L) two tomato plants monoculture]
579 under no P added and 120 mg·kg⁻¹ P added treatments. The contour lines are at intervals of 1 g
580 root fresh weight per kilogram fresh soil.
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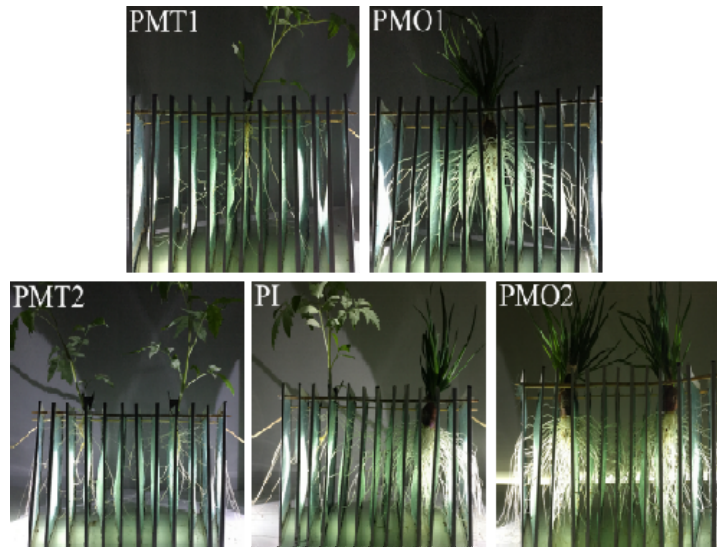
583 **Fig. 3.** Images of roots from different tomato/potato onion combinations under no P added
584 conditions at day 12. (P0MT1) one tomato plant in monoculture, (P0MO1) one potato onion
585 plant in monoculture, (P0MT2) two tomato plants in monoculture, (P0I) tomato/potato onion
586 intercropping system, (P0MO2) two potato onion plants in monoculture.



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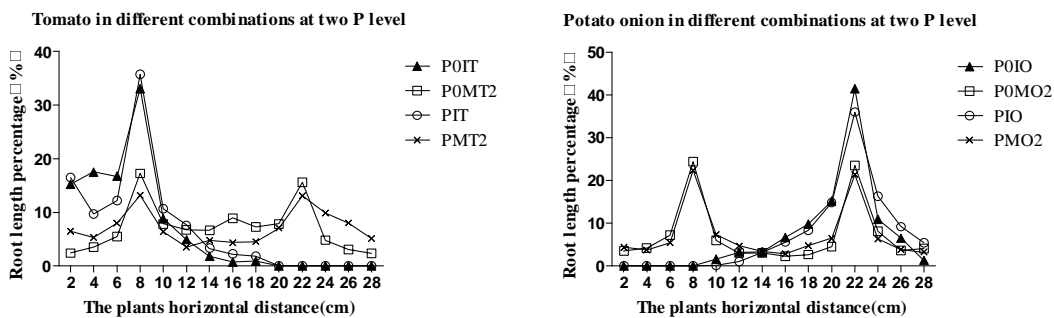
588 **Fig. 4.** Images of roots from different tomato/potato onion combinations under 120 mg·kg⁻¹ P
589 added conditions at day 12. (PMT1) one tomato plant in monoculture, (PMO1) one potato onion
590 plant in monoculture, (PMT2), two tomato plants in monoculture, (Zhang et al.) tomato/potato
591 onion intercropping system, (PMO2) two potato onion plants in monoculture.

592



593

594 **Fig. 5.** Root percentage data for tomato and potato onion grown in different combinations in 14
 595 spaces. The distribution of the tomato root percentage is shown on the left and that of the potato
 596 onion root percentage is shown on the right. (P0IT) tomato in the intercropping system under no
 597 P added, (P0MT2) tomato monoculture under no P added, (PIT) tomato in the intercropping
 598 system under $120 \text{ mg}\cdot\text{kg}^{-1}$ P added, (PMT2) tomato monoculture under $120 \text{ mg}\cdot\text{kg}^{-1}$ P added;
 599 (P0IO) potato onion in the intercropping system under no P added, (P0MO2) potato onion
 600 monoculture under no P added, (PIO) potato onion the in intercropping system under $120 \text{ mg}\cdot\text{kg}^{-1}$
 601 ^1P added, (PMO2) potato onion monoculture under $120 \text{ mg}\cdot\text{kg}^{-1}$ P added.



602

603 **TABLES**

604 **Table 1.** Effect of intercropping with potato onion on tomato's plant growth (mg plant⁻¹) at
 605 different P levels.

	P0		P120		F-statistics		
	Monocultu re	Mixed culture	Monocultu re	Mixed culture	P level	Cropping pattern	P levelx Cropping pattern
Shoot dry weight (mg shoot ⁻¹)	0.73±0.05 b	0.99±0.02 a	1.20±0.08 b	1.65±0.07 a	263.83***	104.56***	7.61*
Root dry weight (mg root ⁻¹)	0.18±0.02 a	0.21±0.01 a	0.17±0.01 a	0.20±0.03 a	0.39	7.47*	0.09
Total plant dry weight (mg plant ⁻¹)	0.91±0.04 b	1.20±0.02 a	1.37±0.08 b	1.85±0.09 a	214.12***	102.80***	5.89*

606
 607 P0 represents no P added treatment, P120 represents 120 mg·kg⁻¹ P added treatment. *, **, ***
 608 represent sterilization contrasts significant at the 0.05, 0.01, and 0.001 probability levels,
 609 respectively.