1   *Short title:* **Synergy of shallow roots and localized P supply**

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5   **Synergy between a shallow root system with a *DRO1* homologue and localized P**
6   **application improves rice P uptake**

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14   *Author contributions:* A.Z.O., and Y.T. designed research and analyzed data; A.Z.O., Y.T.,

15   M.M., and T.N. performed research; A.Z.O., and Y.T. wrote the article with contribution of all

16   authors; Y.U. developed and provided plant materials; All authors reviewed, revised and

17   approved the articles; Y.T. agrees to serve as the author responsible for contact and ensures

18   communication.

19   *One sentence summary:* A combination of a micro-dose, localized phosphorus (P) application

20   and a shallow root system improves rice P uptake which can increase crop production with

21   reduced environmental impacts.

26

ABSTRACT

The development of genotypes and fertilizer management practices that facilitate high phosphorus (P) use efficiency is needed given the depleting phosphorus ore deposits and increasing ecological concerns about its excessive use. Root system architecture (RSA) is important in efficiently capturing immobile P in soils, while agronomically, localized P application near the roots is a potential approach to address this issue. However, the interaction between genetic traits of RSA and localized P application has not been examined. Near-isogenic lines (NILs) and their parent of rice (*qsor1*-NIL, *Dro1*-NIL, and IR64, with shallow, deep, and intermediate root growth angles (RGA), respectively) were grown in flooded pots in a uniform and P-sufficient condition (P_{inco}), and with localized P application by dipping seedling roots into P-enriched slurry at transplanting (P-dipping). The P-dipping created an available P hotspot at the soil surface and substantially improved applied P-use efficiency (equivalent biomass at one fifth of application rate of the P_{inco}). Further, the *qsor1*-NIL had significantly greater biomass and P uptake than the other genotypes in the P-dipping. The *qsor1*-NIL consistently had a greater root biomass and surface area in the 0–3 cm soil layer, despite that there were no genotype differences in total values and that the other genotypes also reduced their RGAs responding to the P hotspot in the P-dipping. The shallow root system of *qsor1*-NIL facilitated P uptake from the P hotspot. P-use efficiency in crop production can be further increased by combining genetic traits of RSA and localized P application.

Keywords: near-isogenic line, *Oryza sativa* L., phosphorus deficiency, P-dipping, root system architecture (RSA), root plasticity, root growth angle (RGA)

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INTRODUCTION

50   Phosphorous deficiency restricts crop growth, particularly in the tropics, due to the inherently

51   low P content of soils and the high P-fixing capacity of other minerals such as active Al- and

52   Fe- oxides (Walker and Syers 1976). Large amounts of mineral P fertilizer have been

53   continuously applied to overcome low P-use efficiency and achieve high grain yields. Given the

54   finite nature of the P fertilizer resource and increasing ecological concerns about the excess use

55   of P in agricultural systems (Vance et al. 2003; Carpenter and Bennett 2011; Nedelciu et al.

56   2020), it is vital to investigate sustainable crop production strategies that facilitate the efficient

57   utilization of applied and available P in soils. Such strategies are also critical for the food

58   security of resource-poor farmers with low fertilizer inputs in developing countries (Tsujimoto

59   et al. 2019).

60   Roots play a pivotal role in exploring immobile P in the soil. An increased root surface area

61   with minimal carbon costs is one strategy, through the formation of finer roots, aerenchyma,

62   and root hairs (Lynch and Ho, 2005; Nestler et al. 2016; Lynch 2019). Changes in root system

63   architecture (RSA) such as the development of surface roots is another root function to adapt to

64   P deficiency, that is so called topsoil foraging, because P is most available in surface soil layers

65   (Lynch and Brown, 2001). This topsoil foraging can be enhanced by a shallower growth angle

66   of axial roots (Lynch and Brown, 2001), adventitious root abundance (Miller et al. 2003), and

67   many/short lateral root branching (Jia et al. 2018). Field-based studies have demonstrated the

68   yield advantages of genotypes with these architectural traits for several crops under

69   P-deficiency (Lynch 2019). Therefore, identification of key root traits and their genetic

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70   mechanisms and conferring genes or quantitative trait loci (QTL) should offer avenues for

71   improving P acquisition efficiency in crop breeding (Burridge et al. 2019).

72   Agronomic approach for improving P-use efficiency includes localized fertilization, which

73   refers to the placement of small amounts of fertilizers nearby the root zone. Several field

74   experiments have demonstrated the positive impacts of localized P fertilization on grain yields

75   and/or fertilizer use efficiencies for crop production (e.g., Vandamme et al. 2018). Our recent

76   study identified that applied P-use efficiency can be substantially improved by dipping seedling

77   roots in P-enriched slurry at transplanting (P-dipping) in severely P-deficient rice fields in

78   Madagascar (Rakotoarisoa et al. 2020). The P-dipping transfers P, with the slurry attached to

79   seedling roots, creating a soluble P hotspot nearby the transplanted roots and facilitating plant P

80   uptake, even under the high P-fixing soils of the tropics (Oo et al. 2020). The use of P-dipping

81   is currently being tested by hundreds of smallholder farmers in Madagascar.

82   Despite a range of studies in both genetic and agronomic approaches, none have examined how

83   the combination of RSA traits and localized fertilization would affect plant P-use and

84   acquisition efficiencies. In the present study, we aimed to identify the combination effect by

85   using near-isogenic lines (NILs) of *DRO1* and its homologue (*qSOR1*), the major QTLs of rice

86   controlling root growth angle (RGA). The parent variety, IR64, is a high-yielding, modern

87   variety with a relatively shallow RGA with the combination of the nonfunctional allele of

88   *DRO1* and the functional allele of *qSOR1*. The *Dro1*-NIL, developed by Uga et al. (2013), has a

89   relatively deep RGA with the combination of functional alleles of both *DRO1* and *qSOR1*. The

90   *qsor1*-NIL, developed by Kitomi et al. (2020), has a shallower RGA than IR64, with the

91   combination of nonfunctional alleles of both *DRO1* and *qSOR1*. We hypothesize that P-dipping,

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92   creating the P hotspot at the soil surface, will have a positive interaction with the shallow root

93   system in rice. By understanding the interaction, further research can be expected to improve

94   applied P use efficiencies by designing RSA traits for localized fertilizer application techniques.

95

96   **RESULTS**

97   Localized P application via P-dipping (P_{dip}) achieved equivalent biomass and P uptakes at one

98   fifth of the application rate of uniform P incorporation (P_{inco}) (Fig. 1). The ANOVA detected

99   consistent and significant interactions between genotype and P treatment for shoot biomass and

100 P uptakes at both 21 days after transplanting (DAT) and 42 DAT. In the P_{dip} treatment,

101 *qsor1*-NIL consistently had greater shoot biomass and P uptake than *Dro1*-NIL. In contrast, in

102 P_{inco}, *Dro1*-NIL tended to have greater shoot biomass and significantly greater P uptakes than

103 the other genotypes at 42 DAT. Applied P-use efficiency (calculated as the ratio of shoot P

104 uptake at 42 DAT to the amount of P applied) increased from 3.4% to 16.2% for IR64 by

105 changing the P application methods from P incorporation to P-dipping and further increased to

106 20.0% by using *qsor1*-NIL (data not shown).

107 The RSA traits among genotypes were consistent under P_{dip}: the RGA was the shallowest in the

108 order of *qsor1*-NIL > IR64 > *Dro1*-NIL at both 21 DAT and 42 DAT (Fig. 2). As a result of the

109 RGA differences, *qsor1*-NIL developed a large proportion of root biomass and root surface area

110 in the 0–3 cm layer and little in the 14–28 cm layer. In contrast, *Dro1*-NIL distributed a

111 relatively large proportion of root mass in the 14–28 cm layer. For instance, at 21 DAT,

112 *qsor1*-NIL developed 50.3% of the root mass in the 0–3 cm layer and only 2.0% in the 14–28

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113   cm layer while these proportions were 32.7% and 10.3% for *Dro1*-NIL. The root distribution

114   pattern of IR64 was intermediate between *qsor1*-NIL and *Dro1*-NIL. The trend in RSA among

115   genotypes were the same in P_{inco} while IR64 and *Dro1*-NIL tended to have deeper RGAs than

116   those in P_{dip} (Fig. 3). The RGAs of *qsor1*-NIL, IR64, and *Dro1*-NIL at 21 DAT were 7.1°, 23.6°,

117   and 33.3° in P_{dip} and 5.0°, 39.8°, and 52.2° in P_{inco}.

118   By reflecting the differences in RSA, *Dro1*-NIL had a greater root biomass, greater root surface

119   areas, and longer lateral and nodal root length than the other genotypes in the 14–28 cm layer

120   (the difference was only significant vs. *qsor1*-NIL), despite its significantly lower values in the

121   total for these parameters at 21 DAT (Fig. 4). At 42 DAT, there were no significant differences

122   in the total values of these parameters except nodal root length, whereas genotype root

123   distribution patterns were retained within each soil layer: the *qsor1*-NIL had significantly

124   greater root mass, greater root surface area, and longer nodal root length than *Dro1*-NIL in the

125   0–3cm layer and vice versa in 14–28 cm (Fig. 4). IR64 was intermediate for these parameters in

126   both the 0–3 cm and 14–28 cm layers.

127   Soluble P concentrations in soils were averaged across genotypes because there were no

128   significant genotype differences in any sampling times or sampling layers. The P_{dip} had a

129   substantially large soluble P concentration at a depth of 3 cm (Fig. 5). The maximum P

130   concentration at a depth of 3 cm for P_{dip} was >100 times greater than the other depths for both P

131   treatments throughout the growing period. In P_{dip}, soluble P concentrations were greater at a

132   depth of 7 cm than at 21 cm in the latter growth stages, but apparently the vertical P diffusion

133   from the 3 cm hotspot was relatively small. In contrast, the soluble P concentrations were

134   significantly higher at a depth of 21 cm than at 7 cm in P_{inco} after 28 DAT.

135

136 **DISCUSSION**

137 The results support the hypothesis that the shallow root system of *qsor1*-NIL has a positive

138 interaction with localized P application via P-dipping and that the combination additively

139 improves applied P-use efficiency for initial rice growth. The other genotypes also reduced the

140 RGA by 16–19° in response to the P hotspot (Fig. 2, 3), yet the synergy with P-dipping was

141 greater in *qsor1*-NIL. This implies that breeding efforts to design the RGA in localized P spots

142 can be more beneficial than relying on the intrinsic root plasticity of each genotype.

143 Superior P uptake of *qsor1*-NIL with P-dipping is attributable to the greater root biomass and

144 root surface area in the 0–3 cm soil layer where high soluble P is available throughout the

145 growing period. This is most likely the same mechanism as topsoil foraging, prioritizing the

146 root development in the P-rich domains to efficiently capture immobile P in soils.

147 Spatio-temporal P variations in the P-dipping indicate that applied P mobility is highly

148 restricted despite a general understanding that P becomes less immobile under flooded

149 conditions (Turner and Gilliam, 1976), emphasizing the importance of RSA for the localized P

150 acquisition, even under flooded soil culture. The effect of topsoil foraging itself has been

151 reported in several upland crops (Zhu et al. 2005; Miguel et al. 2015; Jia et al. 2018; Sun et al.

152 2018), but not in rice. Previous studies detected no significant effects of root distribution

153 patterns or RGA for rice P acquisition under P deficiency (e.g., Mori et al. 2016), which may be

154 due to the materials differing not only in root system architecture but in other traits or in more

155 complex screening environments. The present study had an advantage using NILs differing in

156   RGAs otherwise equivalent phenotypes (Kitomi et al. 2020) under non-water-stressed and

157   greatly uneven P availability by P-dipping.

158   In addition, the present study detected a positive effect of *Dro1*-NIL for P uptake under uniform,

159   P-sufficient conditions. The reason for this positive interaction should be further explored but

160   can be related to consistent P acquisition from the P-rich subsoil layers after the depletion of

161   available P in topsoil layers (Fig. 5). Another potential reason is the more efficient acquisition

162   of other nutrients, such as N, which is vertically more mobile than P. Deep rooting has been

163   reported as a positive trait for N acquisition of upland crops (Lynch, 2019) and also of rice in

164   flooded paddy fields in the latter growth stages (Arai-Sanoh, 2014). In common bean,

165   Rangarajan et al. (2018) postulated that the greater vertical range of roots with deeper RGA and

166   greater number of basal root whorls is advantageous for biomass production when both N and P

167   are deficient. Likewise, dispersed root distribution of *Dro1*-NIL might have benefited from

168   relatively uniform nutrient conditions of the P_{inco} treatment. *Dro1*-NIL had significantly smaller

169   coefficient of variations across soil layers in root biomass at 42 DAT than *qSOR1* (23% vs.

170   47%), indicating more uniform and dispersed root development.

171   It should be noted that crop production environments are complex with multiple abiotic stresses,

172   particularly on smallholder farms in developing countries where stress-resilient and

173   nutrient-efficient technologies are most needed. With this respect, field-based experiments to

174   maturity are further required to confirm the combination effect between genetic RSA traits and

175   P fertilizer management practices. The combination of shallow roots and localized P application

176   can never be a silver bullet. A careful selection of field environments where P deficiency is the

177   primary limiting factor is needed to effectively apply this combination, ideally together with the

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178 development of bimodal root phenotypes (shallow and deep) against complex growing

179 environments. In rice, *qSOR1* and *DRO1* can be promising genetic resources for the

180 development of such bimodal root phenotypes, as indicated by previous studies (Rose et al.

181 2013; Uga et al. 2015).

182

CONCLUSION

184 The study provides a significant evidence by using NILs differing in their RGA that a shallow

185 root system has a positive interaction with localized P application nearby the root at

186 transplanting, and the combination substantially improves applied-P use efficiency for initial

187 rice growth. This finding should encourage relevant research focusing not only on physiological

188 root traits or agronomic management approaches, but on their combination to address to the

189 global issue of increasing crop production while minimizing the environmental impacts.

190

MATERIALS AND METHODS

Experimental design and treatments

193 The experiment was conducted in a greenhouse with an automatic ventilation system at the

194 Japan International Research Center for Agricultural Science (JIRCAS), Tsukuba, Japan. The

195 average daytime and nighttime temperatures during the experiment ranged from 26.2˚ to 35.8˚C

196 and 24.7˚ to 28.7˚C, respectively (Thermo Recorder TR-50U2, T&D Corporation, Japan).

197 The soil for the experiment was collected from a subsoil layer (40–50 cm in depth) at the JIRCAS

198 Tropical Agricultural Research Front, Okinawa, Japan. The soil was sandy clay and had low pH

199 (H_2O) of 4.86, low available P content, and high P retention capacity with abundant active Al and

200 Fe oxides. The soil was air-dried and passed through an 8 mm sieve prior to the experiment.

201 Two different P treatments (sufficient P incorporation (P_{inco}) and localized P application via

202 P-dipping (P_{dip})) were factorially combined with three rice genotypes in a randomized complete

203 block design with seven replications. For both treatments, NH_4NO_3 and K_2SO_4 were mixed with

204 soils and puddled in a bucket at a rate of 220 mg N box^{-1} and 220 mg K_2O box^{-1} to develop uniform

205 and N- and K-sufficient conditions. For the P_{inco}, triple super phosphate (TSP) was added at

206 puddling. Then, the mixed soils were filled into a root box at a rate of 500 mg P_2O_5 box^{-1} to develop

207 a uniform and P-sufficient condition. The root box was made of transparent acrylic sheets with a

208 size of 30 cm height $\times$ 30 cm length $\times$ 3 cm width. The soil was added to the box to a depth of 28

209 cm.

210 For the P_{dip} treatment, a P solution was placed in a spot nearby the transplanted root zone to apply

211 the exact amount of P in all boxes. We estimated the amount of P-enriched slurry transferred or

212 attached to seedling roots at transplanting as 90 mg P_2O_5 box^{-1} based on our previous study (Oo et

213 al., 2020). After the N- and K- added soil was filled in the root box, 90 mg P_2O_5 as TSP dissolved in

214 20 ml water was injected into the soil at a depth of 3 cm in the center of the root box. On the same

215 day of these P treatments, one 10-day old seedling was transplanted in the middle of each root box

216 and grown under continuously flooded conditions.

217 **Measurement**

218 Soil solution samplers (DIK-8393, Daiki Rika Kogyo Co. Ltd., Japan) were installed in one side

219 of the acrylic board in the middle of the 3 cm, 7 cm, and 21 cm depths for four out of seven

10

220   replicates. Soil water samples were collected at 3, 7, 14, 21, 28, and 35 DAT. The samples were

221   analyzed for soluble P concentration using a microplate reader spectrophotometer at an

222   absorbance of 630 nm by following the Malachite Green method (Motomizu et al., 1983).

223   Three and four replicates were harvested at 21 DAT and 42 DAT, respectively. At each harvest

224   time, shoots were cut at ground level and oven-dried at 70 °C for > 48 h to determine shoot

225   biomass. Shoot P concentration was measured with the molybdate blue method (Motomizu et

226   al., 1983) after dry-ashing at 550 °C for 2 h and digestion with 0.5 M HCl. Shoot P uptake was

227   calculated by multiplying the P concentration and shoot biomass.

228   After shoots were removed, root samples were collected using pin-board method as per

229   Kano-Nakata et al (2012). In brief, roots were pinned with a 5 mm mesh net and pinboard after

230   which soils were washed off and digital images were taken. The RGA was determined from the

231   digital image as the angle from the soil surface to the shallowest nodal root using ImageJ software

232   (Version 1.52a, NIH, USA). The root system was then divided into 12 compartments or into the

233   center and both sides of the 0–3 cm, 3–7 m, 7–14 cm, and 14–28 cm soil layers to assess spatial

234   root distributions. Root length and surface area of each compartment were measured using

235   Epson Pro-selection X980 Scanner and WinRhizo Pro software (Regent Instruments, Quebec,

236   Canada). Roots were classified as lateral roots (< 0.2 mm) (Sandhu et al. 2016) and nodal roots (0.2

237   to 2 mm). Roots of > 2 mm were excluded from the analysis, as they were too large for a single root

238   diameter and most likely occurred as a result of a measurement error. After the morphological

239   analysis, root biomass of each compartment was determined by oven-drying at 70 °C for > 48 h.

240   **Statistical analysis**

241   JMP software (v14.0.0, SAS Institute Inc., Japan) was used to perform the statistical analyses.

242   The treatment means were compared at 5% level of probability using Tukey's HSD test after

243   the single and/or interaction effects of genotypes and P treatment were confirmed by a

244   generalized linear model.

245

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251

Figure legends

Fig. 1 Shoot biomass (A) and shoot P uptake (B) of rice genotypes as affected by different P application methods (P incorporation (P_{inco}) of 500 mg P_2O_5 box^{-1} vs. P-dipping (P_{dip}) of 90 mg P_2O_5 box^{-1}) at 21 days after transplanting (DAT) and 42 DAT. Different letters and ns within each treatment indicate significant and non-significant differences, respectively, among genotypes at 5% using Tukey's HSD test. Error bars represent the standard error of replications. The * and ** indicate that the interaction between genotype (G) and P application method (P) are significant at $P < 5\%$ and $P < 1\%$, respectively.

Fig. 2 Root growth angle (RGA) and proportions of root biomass and root surface area in different soil layers of *qsor1*-NIL, IR64, and *Dro1*-NIL at 21 days after transplanting (DAT) and 42 DAT under the P_{dip} treatment. Different letters in the same soil layer indicate significant differences among genotypes at 5% of Tukey's HSD test. ns: not significant at 5% level.

Fig. 3 Root growth angle (RGA) and proportions of root biomass and root surface area in different soil layers of *qsor1*-NIL, IR64, and *Dro1*-NIL at 21 days after transplanting (DAT) and 42 DAT under the P_{inco} treatment. Different letters in the same soil layer indicate significant differences among genotypes at 5% of Tukey's HSD test. ns: not significant at 5% level.

Fig. 4 Root development in different soil layers and in total of all layers at 21 days after transplanting (DAT) and 42 DAT under the P_{dip} treatment. Different small letters and capital letters indicate significant differences among genotypes in these parameters within each soil layer and in total of all layers, respectively, at 5% of Tukey's HSD test. ns: not significant at 5% level.

13

275   **Fig. 5** Spatio-temporal variations in soluble P concentration as affected by different P

276   application methods. The cross symbols indicate the value at the 3 cm depth of the P_{dip}

277   treatment. The open and closed circles indicate the value at the 7 cm depth of the P_{dip}

278   treatment and P_{inco} treatment, respectively. The open and closed triangles indicate the value

279   at the 21 cm depth of the P_{dip} treatment and P_{inco} treatment, respectively. Data values are an

280   average of three rice genotypes because no significant genotype difference in soluble P

281   concentration was observed at each sampling time. Error bars indicate standard error of

282   replications. Different letters indicate significant differences at 5% using Tukey's HSD test

283   among different soil depths (7 cm and 21 cm) by P application methods. The observation at

284   3cm depth was only conducted in the P_{dip} treatment.
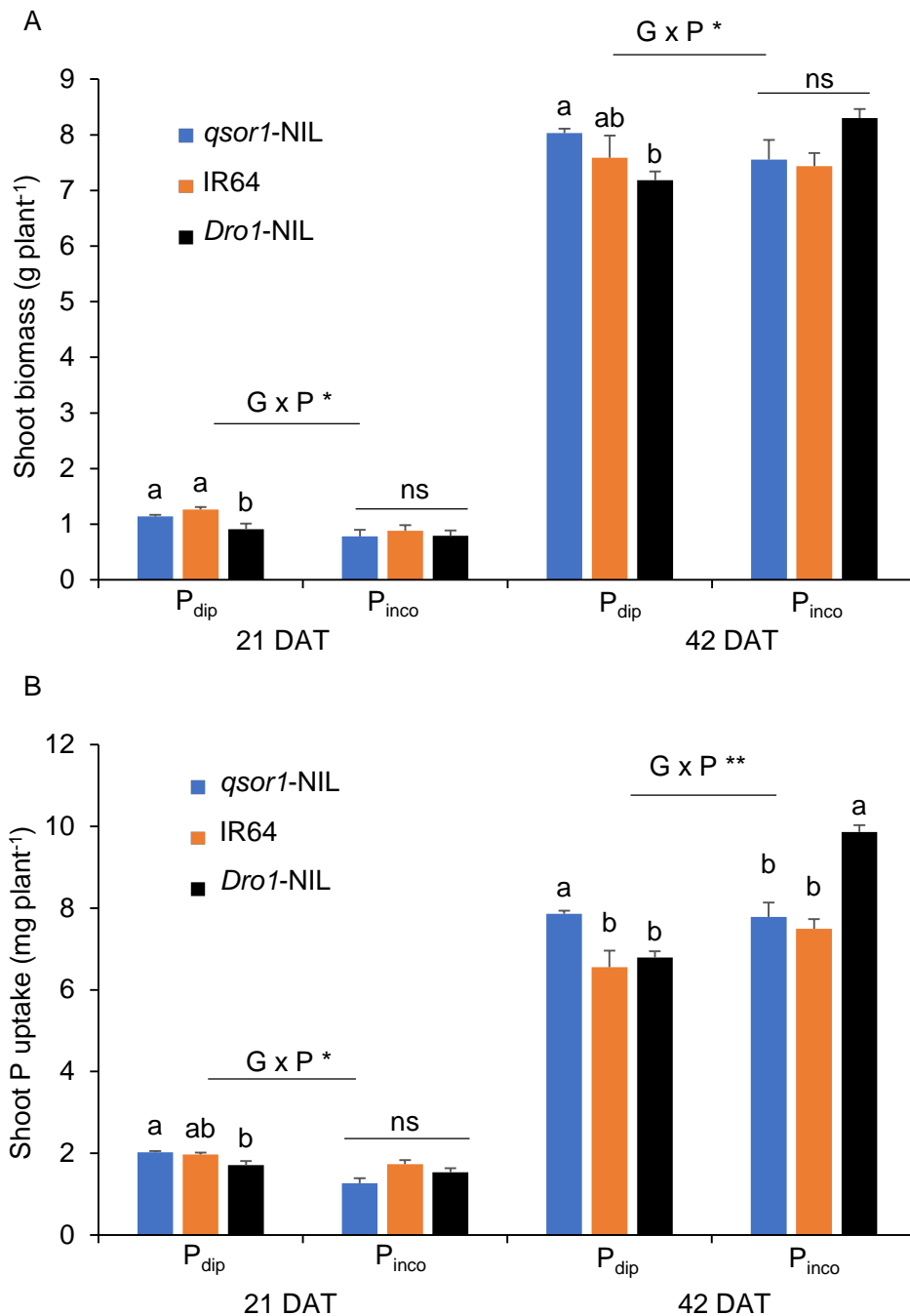
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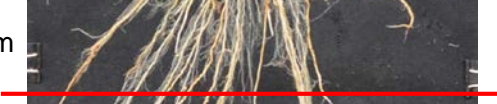
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Fig. 1 Shoot biomass (A) and shoot P uptake (B) of rice genotypes as affected by different P application methods (P incorporation (P_{inco}) of 500 mg P_2O_5 box^{-1} vs. P-dipping (P_{dip}) of 90 mg P_2O_5 box^{-1}) at 21 days after transplanting (DAT) and 42 DAT. Different letters and ns within each treatment indicate significant and non-significant differences, respectively, among genotypes at 5% using Tukey's HSD test. Error bars represent the standard error of replications. The * and ** indicate that the interaction between genotype (G) and P application method (P) are significant at P < 5% and P < 1%, respectively.

	RGA (degree)		Root biomass (%)		Root surface area (%)	
	21DAT	42DAT	21DAT	42DAT	21DAT	42DAT

qsor1-NIL (21 DAT)

Soil layer	RGA 21DAT	RGA 42DAT	Biomass 21DAT	Biomass 42DAT	Surface 21DAT	Surface 42DAT
0-3 cm	7.1b	6.8c	50.3a	43.0a	41.9a	29.1a
3-7 cm			30.1ns	21.3ns	33.9ns	22.4a
7-14 cm			17.7b	21.4c	22.0b	26.2ns
14-28 cm			2.0b	14.4c	2.2b	22.3c

IR64 (21 DAT)

Soil layer	RGA 21DAT	RGA 42DAT	Biomass 21DAT	Biomass 42DAT	Surface 21DAT	Surface 42DAT
0-3 cm	23.6a	20.8b	36.9b	30.1b	28.6b	23.1b
3-7 cm			31.6ns	19.4ns	33.3ns	20.9ab
7-14 cm			26.1a	25.6a	31.2a	27.0ns
14-28 cm			5.4b	24.9b	6.9b	29.0b

Dro1-NIL (21 DAT)

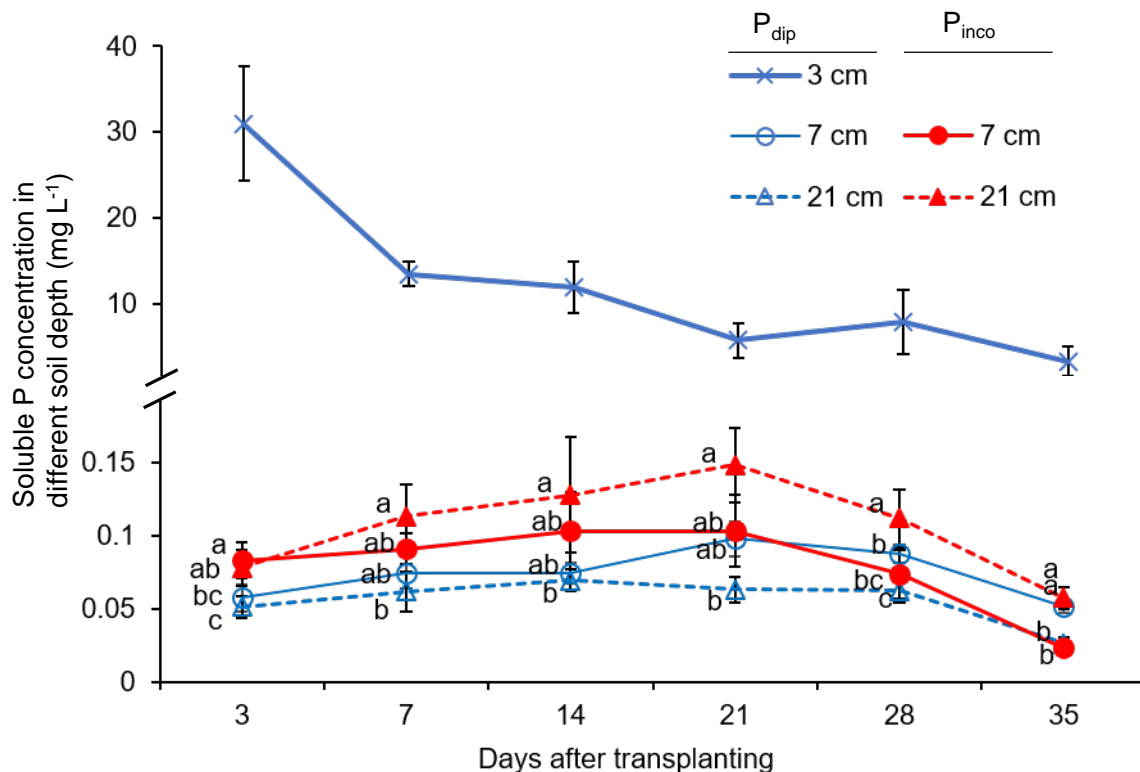| Soil layer	RGA 21DAT	RGA 42DAT	Biomass 21DAT	Biomass 42DAT	Surface 21DAT	Surface 42DAT
0-3 cm	33.3a	30.8a	32.7b	26.9b	26.7b	21.6b
3-7 cm			31.4ns	19.1ns	30.5ns	18.6b
7-14 cm			25.6a	23.6b	30.3a	25.7ns
14-28 cm			10.3a	30.4a	12.5a	34.1a

Fig. 2 Root growth angle (RGA) and proportions of root biomass and root surface area in different soil layers of *qsor1*-NIL, IR64, and *Dro1*-NIL at 21 days after transplanting (DAT) and 42 DAT under the P_{dip} treatment. Different letters in the same soil layer indicate significant differences among genotypes at 5% of Tukey's HSD test. ns: not significant at 5% level.

	RGA (degree)		Root biomass (%)		Root surface area (%)	
	21DAT	42DAT	21DAT	42DAT	21DAT	42DAT
qsor1-NIL (21 DAT)						
0-3 cm	5.0b	4.5c	55.0a	39.9a	43.4a	29.2ns
3-7 cm			31.9ns	25.4a	41.6ns	24.5a
7-14 cm			12.9b	21.5ns	14.7ns	25.7ns
14-28 cm			0.3ns	13.2b	0.3ns	20.7b
IR64 (21 DAT)						
0-3 cm	39.8a	38.0b	39.7ab	39.4a	33.6b	27.4ns
3-7 cm			39.0ns	19.8b	41.4ns	18.8b
7-14 cm			19.4ab	22.8ns	23.2ns	26.5ns
14-28 cm			1.9ns	17.9b	1.8ns	27.4a
Dro1-NIL (21 DAT)						
0-3 cm	52.2a	48.2a	33.5b	30.0b	36.9b	24.3ns
3-7 cm			36.0ns	19.7b	36.5ns	19.9b
7-14 cm			24.3a	23.2ns	21.9ns	25.9ns
14-28 cm			6.2ns	27.1a	4.7ns	29.9a

Fig. 3 Root growth angle (RGA) and proportions of root biomass and root surface area in different soil layers of qsor1-NIL, IR64, and Dro1-NIL at 21 days after transplanting (DAT) and 42 DAT under the P_{inco} treatment. Different letters in the same soil layer indicate significant differences among genotypes at 5% of Tukey's HSD test. ns: not significant at 5% level.
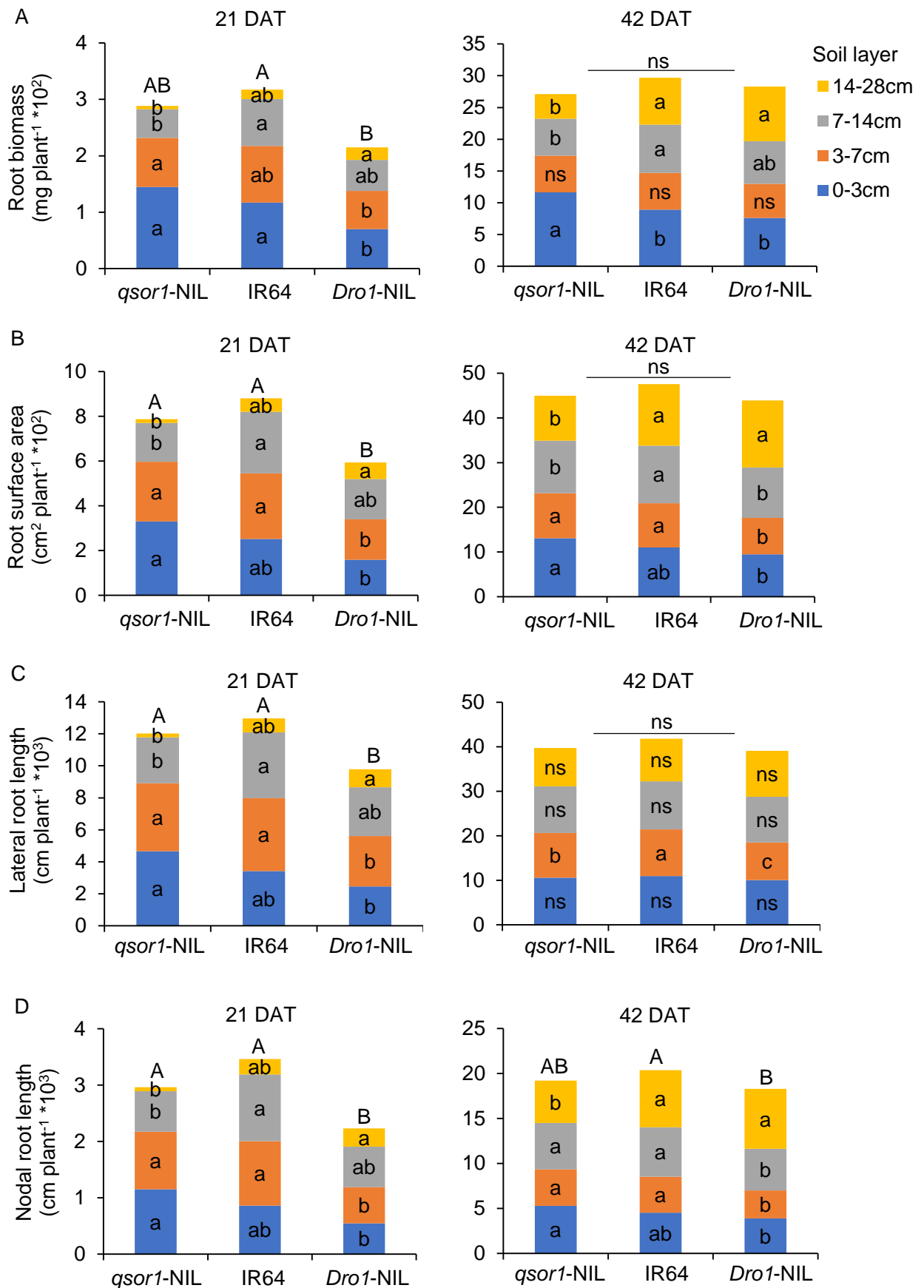
Fig. 4 Root development in different soil layers and in total of all layers at 21 days after transplanting (DAT) and 42 DAT under the P_{dip} treatment. Different small letters and capital letters indicate significant differences among genotypes in these parameters within each soil layer and in total of all layers, respectively, at 5% of Tukey's HSD test. ns: not significant at 5% level.

Fig. 5 Spatio-temporal variations in soluble P concentration as affected by different P application methods. The cross symbols indicate the value at the 3 cm depth of the P_{dip} treatment. The open and closed circles indicate the value at the 7 cm depth of the P_{dip} treatment and P_{inco} treatment, respectively. The open and closed triangles indicate the value at the 21 cm depth of the P_{dip} treatment and P_{inco} treatment, respectively. Data values are an average of three rice genotypes because no significant genotype difference in soluble P concentration was observed at each sampling time. Error bars indicate standard error of replications. Different letters indicate significant differences at 5% using Tukey's HSD test among different soil depths (7 cm and 21 cm) by P application methods. The observation at 3cm depth was only conducted in the P_{dip} treatment.

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