

1 Seed priming enhances early growth and improves area of soil exploration by roots

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8 **Abstract**

9 *Introduction*

10 Seed priming has been conducted for centuries with growth advantages reported for a variety
11 of different crops. Previous work has suggested priming does not offer a yield advantage
12 despite an increased early growth if grown under ideal conditions. However, how these
13 advantages unfold in regards to early root development is largely unknown.

14 *Results*

15 We observed accelerated germination speed in primed seeds regardless of applied seed
16 enhancement technology i.e. coating or pelleting. Additionally, we found significant
17 differences in lateral root development in primed seeds vs non-primed seeds. Furthermore,
18 we recorded an increase in volume and surface of embryo and perisperm indicating a distinct
19 morphological change during the germination process of primed seeds compared to non-
20 primed seeds.

21 *Conclusions*

22 We attribute the enhanced early plant development in primed seeds to increased root
23 development and thus enhanced volume of the soil resource mined for nutrients. This
24 improvement can be detected four days after emergence within the root system throughout
25 the early plant development despite an early transition from seed reserves to soil based
26 growth. The understanding of belowground root architecture characteristics can improve the
27 selection of appropriate seed enhancement technologies and seedbed management practices.

28 **Keywords**

29 Root; Seed; Seedling growth; Seed priming; Soil exploration; X-ray CT

30 **Abbreviations**

31 NUS / NS+: Naked non-primed / Naked primed

32	PUS / PS+:	Pelleted non-primed / Pelleted primed
33	PUSCO / PS+CO:	Pelleted & Coated non-primed / Pelleted & Coated primed
34	X-ray CT:	X-ray Computed Tomography
35		

36 **1. Introduction**

37 The process of seed germination can be divided into three steps; (1) imbibition, (2) activation
38 or lag phase and (3) root protrusion (Rajjou et al., 2012). In centuries of agricultural practise,
39 amendments were developed to improve the seed performance under varying conditions.
40 Physical seed enhancement technologies include magnetic (magnetic fluids used for removal
41 of contaminants), radiation (UV, microwave, ion radiation, X-ray and gamma-ray radiation
42 improve seed vigour but it is unclear how) and plasma (non-thermal plasma reduces pathogen
43 and chemical contamination in seeds) applications. Besides these physiological and physical
44 seed enhancement technologies, the chemistry of the seed can also be modified to increase
45 its vigour (Afzal et al., 2016; Araújo et al., 2016; Evenari, 1984; Sivachandiran and Khacef,
46 2017).

47 Historically, a variety of soaking methods have been reported that are affecting the
48 germination rate e.g. mixtures of water and honey (Gaius, *Naturalis Historia*), manure (Oliver
49 de Serres, 1539-1619) or osmo-priming in sea water (Darwin, 1855) (Paparella et al., 2015).
50 Processes used to initiate the initial phases of germination by supplying a limited amount of
51 water are called 'priming'. The selection of appropriate enhancement techniques is highly
52 dependent on plant species, seed lot, seed vigour and priming procedure (Ellis and Butcher,
53 1988; Hill et al., 2008; Ibrahim, 2016; Paparella et al., 2015). Historical advancements in seed
54 priming technologies led to improvements in emergence uniformity, stress tolerance and yield
55 consistency. A faster and more uniform emergence, a reduced thermal time (accumulated
56 degrees above base temperature), a higher resistance to pathogens, improved competitive
57 ability over weed plants and a better performance under stress conditions (Jalali and Salehi,
58 2013; Paparella et al., 2015) also collectively known as the 'vigour effect' of priming. For
59 weaker plants (e.g. sugar beet) in particular, the ability to compete with weed species is crucial.
60 Besides this competition issues, commonly plant seeds are rarely able to germinate under
61 optimal conditions due to the environmental influences. A uniform establishment can be
62 achieved with priming through the induction of structural modifications diminishing seed
63 water relation differences of individual seeds (Galhaut et al., 2014). An accelerated and

64 uniform establishment with maintaining historic sowing practices lead to a prolonged growing
65 period therefore improving yield (Khan et al., 1983; Lutts et al., 2016).

66 A variety of different priming techniques have been applied to seeds to improve their viability
67 and performance in the field although commercial seed suppliers tend to keep their priming
68 procedure confidential. In general, *advancing* is a basic technique involving imbibition using a
69 limited amount of water to reduce the amount of water necessary for the germination process
70 with a reported increase of 2 to 3% in germination rate for carrot seeds (Austin et al., 1969;
71 Longden, 1971). *Hydropriming* is a similar process involving a partial seed hydration (10 to
72 20% of full hydration) using distilled water to improve the resistance of the seed for example
73 against salinity or drought (McDonald, 2000; Pill, 1995). In *osmo-priming*, osmotic solutions
74 are used to reduce the impact of reactive oxygen species by limiting oxidative damages
75 (Paparella et al., 2015; Taylor et al., 1998). Priming has several advantages especially under
76 stress conditions (Knypl and Khan, 1980; Passam et al., 1989; Pill, 1991; Wiebe and Muhyaddin,
77 1987). For storage purposes, primed seeds undergo a subsequent dehydration process to
78 reduce the moisture content rapidly back to the original content (Rajjou et al., 2012).

79 Reports of negative effects involving seed priming remain rare so that agricultural companies
80 commonly offer seeds in a primed state as in worst case, primed and non-primed seeds would
81 produce similar ultimate yield under optimal conditions.

82 Lutts et al. (2016) described in an extensive literature review microbiological processes
83 influenced during different priming techniques highlighting molecular approaches available
84 for assessing the role of priming. However, they point out that there are unresolved questions
85 on the origin of the growth stimulation. Although the benefits of seed priming have been
86 obvious practically, the exact mode in which seeds perform better under actual agricultural
87 practice has been mostly correlative as the opaque nature of the soil matrix makes it difficult
88 to observe processes *in situ* (Brown et al., 1996). Over the last 30 years, X-ray Computed
89 Tomography (X-ray CT) has become increasingly popular in the agricultural sciences to
90 quantify the structure of the soil matrix, determining factors like porosity (Kravchenko et al.,
91 2014; Rabot et al., 2018) and measure plant root architecture responses to the soil
92 environment (Mairhofer et al., 2013; Tracy et al., 2013). Gregory et al. (2003) was one of the
93 first researchers to use CT to describe the germination of wheat seedlings at a resolution of
94 100 μm . More recently, Blunk et al. (2017) found a significant positive influence of the coating

95 on the growth rate of seedlings in a growth comparison study between physical seed
96 enhancement technologies using X-ray CT (resolution 20 μm).

97 In general, little is known about the physical differences on early growth of primed seeds vs.
98 non-primed seeds. The aim of this study was to determine the influence of the priming process
99 on sugar beet seeds in terms of their *in situ* development and their early growth stage root
100 architecture. X-ray CT was used to non-destructively quantify the growth pattern of both
101 primed and non-primed seeds.

102 **2. Materials & Methods**

103 *2.1. Treatment preparation*

104 A loamy sand soil of the Newport series (83.2% sand, 4.7% silt, 12.1% clay and 2.93% organic
105 matter) was collected from the University of Nottingham farm at Bunny, Nottinghamshire, UK
106 (52.8586°, -1.1280°). Prior to packing, the soil was air-dried and sieved to < 1 mm. Sugar beet
107 (*Beta vulgaris* L.) seed material was supplied by Syngenta Seeds AB. Naked, untreated seeds
108 (NUS) were used alongside woodmeal and clay pelleted seeds (PUS) as well as seeds coated
109 with insecticide and fungicide additionally to the pelleting (PUSCO). Each treatment was
110 available as either primed (NS+, PS+, PS+CO) and non-primed treatment (NUS, PUS, PUSCO).
111 The naked coated treatment was omitted from this study as this treatment is not sold to the
112 end user and therefore of no collective interest. The seed pelleting and coating label, the
113 priming procedure, as well as the precise composition are treated confidentially. Four
114 replicates for each treatment were used in the study.

115 To compare differences in embryo and perisperm size between primed and non-primed seeds
116 an initial high resolution study was conducted on dry seed material outside of soil. Only naked
117 untreated seeds were used for this comparison as the priming treatment is conducted prior
118 to the application of physical enhancement technologies. Individual seeds were scanned using
119 a Phoenix Nanotom X-ray CT scanner (GE Measurement & Control Solutions, Wunstorf,
120 Germany) with an X-ray tube potential energy of 75 kV and a current of 120 μA . The detector
121 collected 1800 projection images (image average and skip were set to 3 and 1, respectively)
122 with a timing of 500 ms for each image. The scan spatial resolution and time were 2.5 μm and
123 64 min, respectively. The reconstruction was performed using *phoenix datos/x rec* (GE
124 Measurement & Control Solutions, Wunstorf, Germany) reconstruction software with a beam
125 hardening correction setting of 6 and an automatic scan optimisation.

126 The column packing was conducted as described in Blunk et al. (2017b). The soil columns were
127 scanned using a Phoenix v|tome|x m 240 kV X-ray CT scanner (GE Measurement & Control
128 Solutions, Wunstorf, Germany). The scans were conducted using an X-ray tube potential
129 energy of 130 kV and a current of 100 μ A. The detector collected 2878 projection images with
130 timing of 250 ms per image (FAST SCAN mode; the sample continuously rotates during image
131 acquisition with no averaging or skip) at a resolution of 20 μ m. To image the full length of the
132 column at maximum resolution, the 'multiscan' module in the acquisition software was used
133 to collect two scans per column resulting in a total scan time of 24 minutes (12 mins per
134 section). Reconstruction was conducted using the *phoenix datos|x rec* reconstruction
135 software with a beam hardening correction setting of 8 and an automatic calculation of the
136 region of interest and scan optimisation. All soil columns were scanned in the same order at
137 each time point to reduce temporal effects.

138 2.2. Soil core transplantation

139 Due to the design of the experiment, each soil core was transplanted to a larger column to
140 enable the highest possible resolution for all scanning days (day 2, day 4 and day 14 after
141 imbibition) as well as to allow enough room for the seedling to grow after day 4. The small
142 polypropylene column was pre-cut lengthways (secured with adhesive tape) and included
143 detachable mesh to enable a non-destructive extraction of the soil core following the first
144 stage of growth. After X-ray CT scanning following four days of growth, the soil core was
145 extracted from the column by detaching the mesh and opening the column along the
146 longitudinal axis (Fig. A.1). The soil core was then placed on top of a layer of 435 g dry soil with
147 a height of approximately half of the height of the large polypropylene column (170 mm height
148 and 76 mm inner diameter). The column was then filled with 405 g dry soil to generate a total
149 bulk density of 1.2 g cm⁻³. The soil column was then saturated and drained afterwards to a
150 gravimetric moisture content of 20% w/w. Growth and moisture conditions were maintained
151 as previously described.

152 The larger soil columns were scanned using a Phoenix v|tome|x m 240 kV X-ray CT scanner
153 (GE Measurement & Control Solutions, Wunstorf, Germany) using an X-ray tube potential of
154 180 kV and a current of 180 μ A. The detector collected 2399 projection images with timing of
155 250 ms per image (FAST SCAN mode; the sample continuously rotates during image
156 acquisition with no averaging or skip) at a resolution of 50 μ m. To image the full length of the

157 column at maximum resolution, the 'multiscan' module in the acquisition software was used
158 to collect two scans per column resulting in a total scan time of 20 minutes (10 mins per
159 section). Reconstruction was performed as described earlier.

160 *2.3. Image analysis*

161 Root and lateral root lengths were determined using the polyline tool in VG StudioMax v2.2
162 (Volume Graphics GmbH, Germany). Embryo and perisperm volume and surface area were
163 calculated automatically after segmentation of each structure to a region of interest (ROI) in
164 VG StudioMax v2.2. The convex hull was automatically calculated using an in-house developed
165 tool for measuring root angle analysis of X-ray CT image data based on the polylines of the
166 root system (PAM 1.5. alpha, unpublished). Seed-soil contact calculations were conducted for
167 the day 2 scans based on the method by Blunk et al. (2017b). Briefly, this involved
168 segmentation of the seed (all inner pores were filled using an open/close morphological
169 operation) and surface determination of the soil aggregates. By dilating the ROI for the
170 aggregates, an overlap between the ROIs for the two materials was created and quantified.

171 *2.4. Statistical analysis*

172 The statistical analysis was performed using a linear mixed effect model in R (RStudio, 3.4.2;
173 R Core Team (2017) on root length as well as lateral architecture parameters based on the
174 effects treatment, scanning time, priming status and seed-soil contact. The linear mixed
175 effects model allowed the repeated measurements on the same experimental units to be
176 analysed correctly. Two models were considered, in the first the experimental units were
177 treated as main-plots in a split plot design, with the repeated observations as sub-plots. In this
178 model the correlation between the random effect for any two observations on the same unit
179 is assumed to be the same. In the alternative model the correlation between any two
180 observations was assumed to be a negative exponential function of the difference in time
181 between them. These two models were compared on the Akaike Information Criterion, and
182 the model with the smallest value of this statistic was selected for further analysis. Radicle
183 length as well as lateral root architecture characteristics were modelled in terms of a fixed
184 effect of batch (treated as a blocking factor) and the main effects and interactions of the
185 treatment (NUS / NS+; PUS / PS+; PUSCO / PS+CO), Priming (presence or absence) and the day
186 of measurement (day 2, day 4 or day 14).

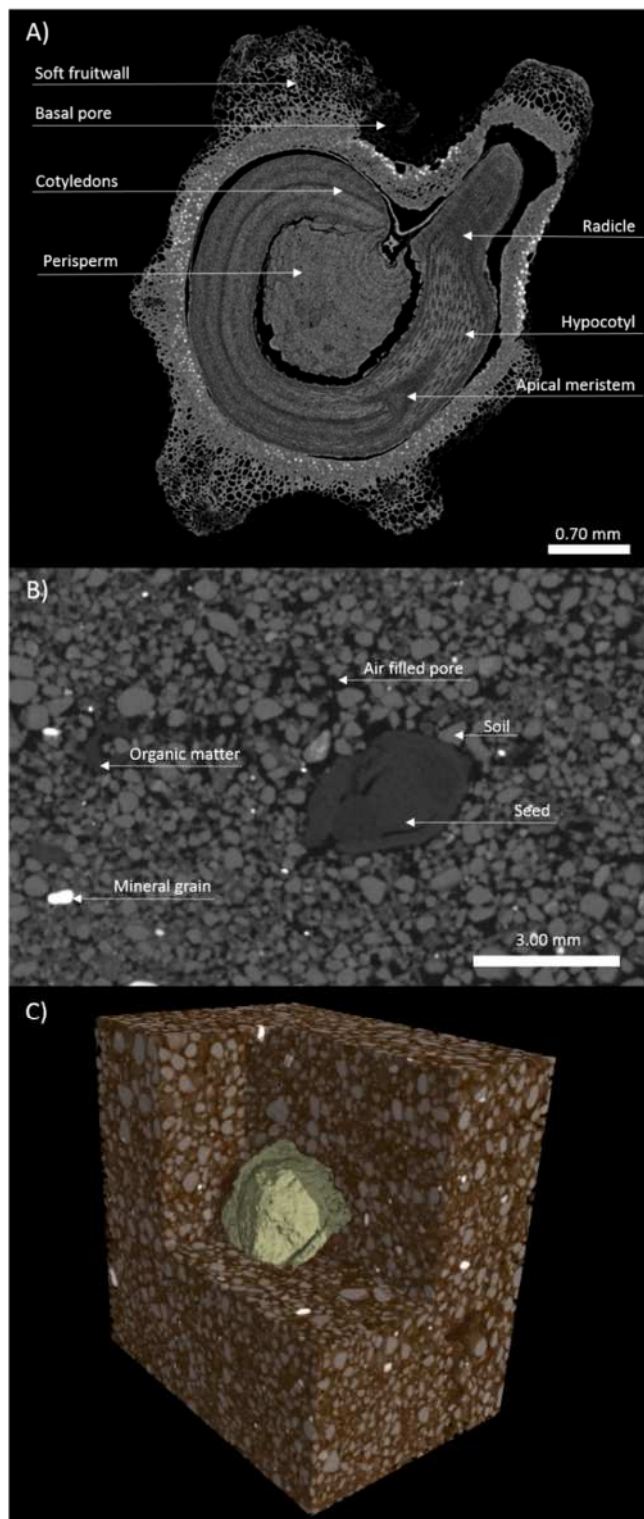
187 The main effect of treatment was partitioned into the following contrasts: Contrast 1 allows
188 us to test the hypothesis that the pelleting procedure does improve radicle growth opposed
189 to naked seeds by testing NUS / NS+ against combined PUS / PS+ & PUSCO / PS+CO
190 measurements. If this contrast were to be significant then the implication is an improved
191 growth behaviour using pelleting technology despite application of coatings containing active
192 ingredients. Contrast 2 tests the hypothesis that the addition of a pesticide coating does
193 impact the growth behaviour by testing the comparison of PUS / PS+ against PUSCO / PS+CO.
194 If this contrast were to be significant, this would imply that the active ingredients in the
195 coating surrounding the pellet do have potential influence on the embryo development. If the
196 interaction of treatment and priming were to be significant, it would indicate that the chosen
197 treatment has an influence on the effect of priming.

198 The main effect of time was partitioned into the following contrasts: Contrast 1 test the
199 hypothesis that the radicle growth in the treatments is of linear nature. If this contrast were
200 to be significant then the implication is a constant growth rate throughout the measured time
201 interval. Contrast 2 allows us to test the hypothesis that the effect of time is on non-linear
202 nature which would imply a non-constant growth if this contrast were to be significant
203 (Wishart and Metakides, 1953). For this instance, orthogonal contrasts for unequal intervals
204 have been used (Snedecor, 1958). If any interaction with the factor time were to be significant,
205 it would indicate a change of effect over time. Similar assumptions have been made for the
206 statistical analysis of lateral root architecture for the day 14 measurements.

207 Additional analyses of variance (ANOVA) were performed using GenStat Seventeenth Edition
208 (Version 17.1.0.14713) analysing the convex hull area. Error bars were calculated as the
209 standard error of the mean.

210 **3. Results**

211 Prior to the growth comparison of primed and non-primed seeds, morphological differences
212 between the treatments were quantified by scanning seeds *ex situ*. The embryo dimensions
213 were assessed as one object as differences in greyscale levels between the organic parts were
214 low and did not allow a distinct separation between cotyledons, hypocotyl and root (Figure 1).



215

216 Figure 1: Quantification of a naked untreated seed. A) 2D X-ray CT image of a bare seed outside
 217 of soil. B) 2D X-ray CT image of a seed within the soil matrix surrounded by aggregates, air and
 218 water filled pores, organic matter and mineral grains. C) 3D rendered X-ray CT image of a
 219 naked seed in soil.

220 No significant differences were found between the primed and non-primed seeds for embryo
 221 volume and surface area as well as perisperm and surface area (Table 1). However, we noted
 222 a trend of increased embryo volume and surface area as well as perisperm surface area in the
 223 primed treatment compared to the non-primed treatment (Table 1).

224 Table 1: Quantification of seed embryo and perisperm volume and surface area. Errors were
 225 calculated as a standard error of the mean.

	Embryo		Perisperm	
	Volume [mm ³]	Surface Area [mm ²]	Volume [mm ³]	Surface Area [mm ²]
NUS	1.65 (± 0.14)	13.85 (± 0.23)	1.05 (± 0.06)	13.45 (± 1.59)
NS+	1.84 (± 0.04)	16.79 (± 1.19)	1.09 (± 0.05)	15.90 (± 1.14)

226

227 The growth behaviour of the seedlings was quantified over time and the transplantation
 228 method appeared successful for all replicates without disturbing the soil matrix. Initial
 229 statistical analysis was performed as a factorial linear mixed effect model and the individual
 230 effects analysed using orthogonal contrasts as displayed in Table 2.

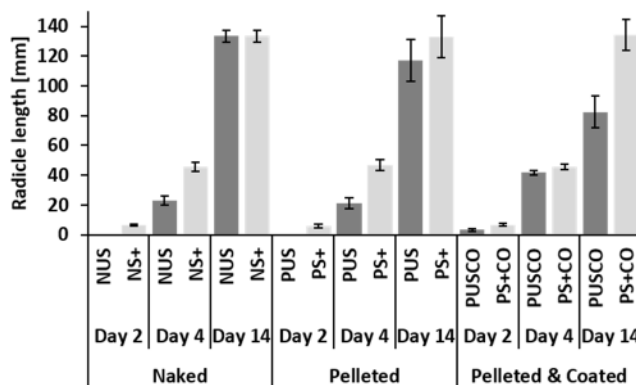
231 Table 2: ANOVA table of factorial analysis including orthogonal contrasts for factors treatment
 232 and day. numDF = degrees of freedom in the numerator; denDF = degrees of freedom in the
 233 denominator; F-value = ratio of variance of group means and the mean of the variances within
 234 the group; p-value = probability; t-value = comparison of sample means to the null hypothesis.

	numDF	denDF	F-value	p-value
Batch	1	27	7.1197	0.0127
Treatment	2	27	2.3910	0.1107
Priming	1	27	12.1960	0.0017
Day	2	56	571.6244	<.0001
Treatment : Priming	2	27	1.5931	0.2218
Treatment : Day	4	56	5.1802	0.0013
Priming : Day	2	56	6.0471	0.0042
Treatment : Priming : Day	4	56	5.3555	0.0010
Contrasts		DF	t-value	p-value
Treatment Contrast 1 (Naked vs Pelleted)		27	1.33	0.196
Treatment Contrast 2 (Pelleted vs Pelleted and coated)		27	0.50	0.618
Day Contrast 1 (Linear)		56	20.84	<0.0001
Day Contrast 2 (Non-linear)		56	3.57	0.0007

235

236 A detailed overview on all analysed contrast interactions is displayed in Table A.1 as Table 2
 237 only gives a simplistic overview on the contrast effects. Naturally, a significant effect of

238 scanning days on root length was observed for both the linear and non-linear effect of time (p
 239 < 0.01). The priming treatment in general had a significant effect on growth ($p < 0.01$). Whilst
 240 the treatment contrasts naked vs treated, and pelleted vs pelleted and coated exhibited no
 241 effect ($p = 0.20$ and $p = 0.62$), the interaction of the treatment contrasts and day was
 242 significantly different for both the linear and non-linear time effects (all four combinations:
 243 $p < 0.01$). A significant interaction of the priming effect with a non-linear time effect was
 244 observed ($p < 0.01$), however not for the linear effect ($p = 0.06$). The threefold treatment,
 245 priming and day effect did also show a significant effect on the root length for all contrasts (p
 246 < 0.01) except for effect of priming on the interaction of the non-linear time effect and the
 247 naked vs pelleting treatments comparison ($p = 0.13$). The growth advantage was observed for
 248 the first two time points (day 2 and day 4) in regards of the tap root length for the primed
 249 treatments in comparison with the non-primed treatments. This behaviour reduced over time
 250 resulting in similar tap root lengths on day 14 (Figure 2 and Figure 3). A difference for PUSCO
 251 vs. PS+CO was found on day 1 and 14 showing a longer root for the primed treatment,
 252 however, not on day 4 (Figure 2).

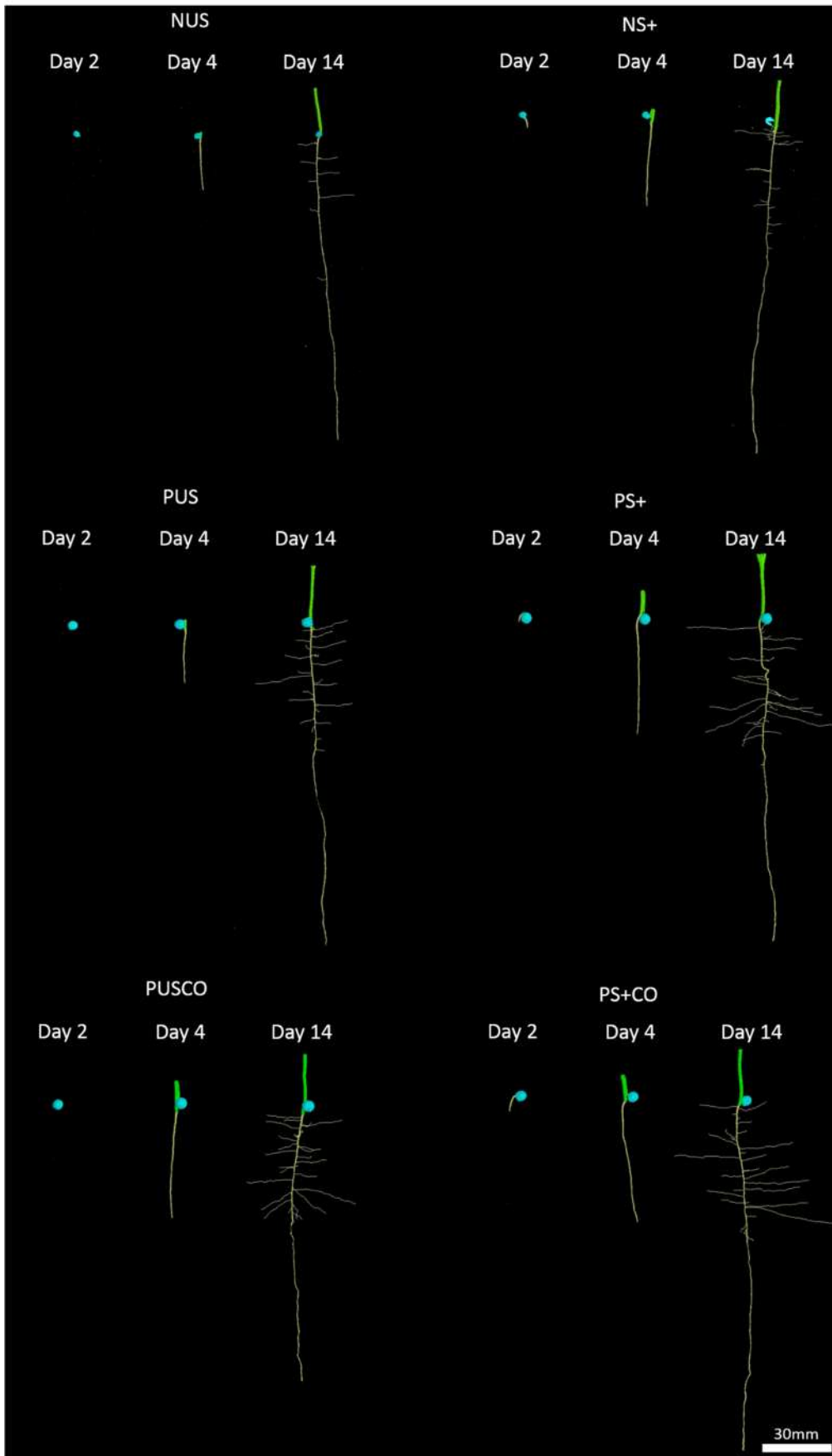


253

254 Figure 2: Root growth of sugar beet seedlings over time showing three treatment comparisons
 255 as pairs (dark: non-primed; light: primed). Error bars calculated as a standard error of the
 256 mean.

257 A difference in 3D root system architecture was observed showing a higher number and
 258 increased length of lateral roots for the primed treatments (Figure 3).

259

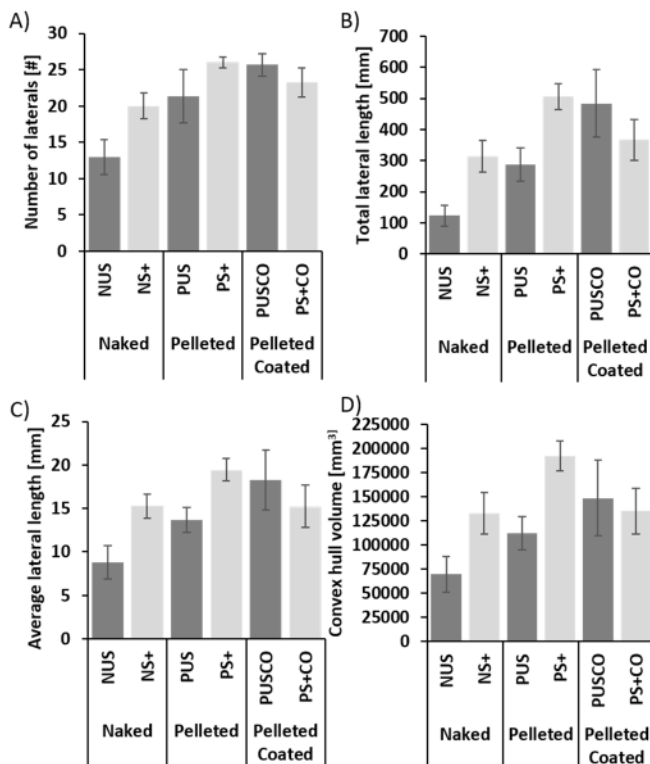


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261 Figure 3: Temporal growth representation of one representative seedling per treatment.

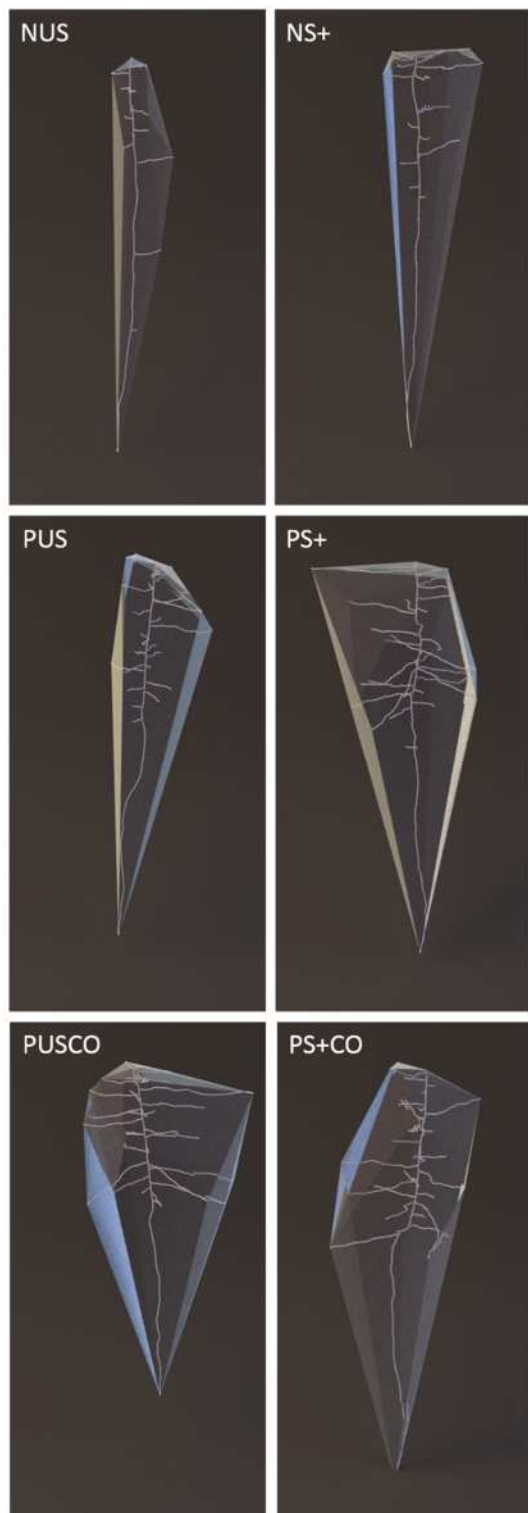
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263 A significant effect of the pelleting treatments in comparison for the naked seed ($p = 0.01$) was
264 observed for the average lateral root length (Figure 4C), calculated as the ratio of total lateral
265 length (Figure 4B) and number of laterals (Figure 4A). However, no significant effect of priming
266 was found with regards to the average lateral root length ($p = 0.10$) (Table A.2). The difference
267 in root architecture was furthermore quantified using the convex hull (smallest convex object
268 set containing all roots). A significantly larger convex hull was observed for the treated seeds
269 in comparison to the naked seed ($p = 0.03$) whereas both pelleted treatments exhibited no
270 significant difference in convex hull size ($p = 0.16$). In general, a significantly increased convex
271 hull was observed for priming ($p = 0.02$). The combined effect of priming and the contrast of
272 treated seeds (pelleted vs pelleted and coated) exhibited a significant difference ($p < 0.01$) in
273 contrast to the comparison of the naked and the treated seeds (Figure 4D and Figure 5).



274

275 Figure 4: Growth comparison of lateral roots after 14 days of growth. A) Number of laterals
276 counted. B) Total lateral length calculated as accumulative length of all laterals. C) Average
277 length calculated as a ratio of total lateral length divided by number of laterals. Error bars
278 calculated as standard error of the mean.



279

280 Figure 5: Convex hull of a representative root system from each treatment presented in Figure
 281 4. The smallest convex object set surrounding 100% of the length of all lateral roots and the
 282 primary root.

283 Calculations for seed-soil contact based on Blunk et al. (2017b) showed no significant
 284 interactions between the contact area and growth characteristics (e.g. tap root length and

285 lateral growth) as well as treatment information (e.g. priming or pelleting) due to a high
286 variability within the dataset. A regression between the seed-soil contact on day 2 and the
287 root growth rates was fitted separately (Fig. A.2). In general, seed-soil contact did not
288 correlate with growth rate at any of the three time points measured ($p = 0.54$). The R^2 values
289 for all treatments showed a low conformity of the fitted regression line to the data points
290 except for PS+ on day 4 with an R^2 of 0.80 showing an increased growth rate with rising contact
291 area. A negative trend was observed for NS+ on day 2 with an R^2 of 0.63 exhibiting a decrease
292 growth rate with increasing seed-soil contact.

293 **4. Discussion**

294 A common assumption of the seed priming process is that biological processes are initiated
295 inducing all metabolic activities necessary for germination, however almost no morphological
296 differences occur as they are irreversible (Hill, 1999). Although no significant differences were
297 observed in the volume and surface area of seed embryo and perisperm in this study, a
298 positive trend was detected that could suggest swelling of these structures during the
299 germination process in primed seeds which was also described earlier for Parsley
300 (*Petroselinum crispum*) and cabbage (*Brassica oleracea*) (Olszewski et al., 2005; Sakata and
301 Tagawa, 2009). Other work however suggested no change in embryo volume for allium species
302 like leek and onion upon priming, however a change was detected for carrot under the same
303 conditions (Gray et al., 1990). Based on the present data we are inclined to support the view
304 of an increase in embryo volume by priming for sugar beet.

305 The primed seeds had a significantly faster growth rate over the first four days compared to
306 non-primed seeds which agrees with previous findings stating a uniform and accelerated
307 germination using varying priming techniques (Paparella et al., 2015). Furthermore, as
308 significant combined effects were found for priming, treatment and day using the linear mixed
309 effect model it highlights the growth advantages of seed priming regardless of the applied
310 physical enhancement. In general, the utilisation of seed storage reserves diminishes upon
311 seedling growth by a shift from a hetero- to an autotrophic metabolism (Bewley and Black,
312 1994). The direct impact of seed pelleting applications on seedling growth is therefore
313 disrupted upon disconnection of the seedling from the seed transitioning to a soil nutrient-
314 based growth which was observed for most of the seedlings between day 2 and day 4 after
315 imbibition. This disconnection highlights the limited amount time pelleting compositions pose

316 influence on the seedling. Primed seeds have been reported to have a similar ultimate yield
317 under ideal conditions compared to a non-primed treatment supporting our observation of
318 similar tap root lengths after 14 days of growth which might also be an artefact of restricted
319 growth due to the vessel size (Danneberger et al., 1992). Also, the number of basal roots was
320 reported as being similar for pepper after 14 days of growth agreeing with our findings of no
321 significant differences in number of lateral roots upon priming (Stoffella et al., 1992).
322 Furthermore Leskovar and Cantliffe (1993) found no yield difference for primed and non-
323 primed bell pepper seedlings after 50, 70 and 90 days of growth. These descriptions agree
324 with our findings of root measurements both for tap root and lateral root growth during the
325 later growth stage of 14 days indicating a trend to tap root length similarity. Four days after
326 germination, seedlings photosynthesize and are not dependent on seed storage reserves, the
327 soil-based growth reduced the differences in tap root length towards day 14. Due to the
328 limitation of the column height, the growth strategy of the primed seedlings shifted from a
329 deep tap root towards an extensive lateral root system as observed for roots hitting
330 compacted layers or similar obstructions (Idowu and Angadi, 2013), therefore increasing the
331 explored volume which is reflected in the significantly increased root system convex hull for
332 the naked and pelleted primed treatments. This behaviour was observed in all treatments
333 regardless whether a seed enhancement was applied or not. A deviation from this behaviour
334 is posed by the PUSCO treatment that explored a larger volume without reaching the limiting
335 height of the column on day 14 in all replicates. This behaviour can be attributed to an
336 obstruction (i.e. the root apical tip reaching the mesh at the bottom of the column before
337 transplanting) at an earlier growth stage resulting in a shift in growth behaviour. The other
338 non-primed treatments (NUS and PUS) in comparison explored a greater depth before
339 investing into root system area of exploration. The greater convex hull of the primed
340 treatments can be interpreted as an increased area for nutrient accessibility and therefore a
341 more robust growth under limiting conditions.

342 All seed enhancement treatments showed a high variability in seed-soil contact despite a
343 uniformly prepared seedbed which we attribute to the later time point used for calculating
344 the seed-soil contact compared to Blunk et al. (2017b). Upon opening of the seed on day 2,
345 soil aggregates around the seed surface might shift and the soil matrix could get reorganised,
346 therefore increasing the contact percentage artificially as larger contact percentages
347 compared to the earlier studies have been found.

348 **5. Conclusions**

349 A priming treatment is applied prior to the application of physical seed enhancement
350 technologies, altering seed morphology. The tendency for earlier germination of the primed
351 treatment was observed in all treatments despite the application of morphological
352 enhancements, ensuring uniform establishment even under harsh conditions. Although
353 reports indicated similar yield under favourable conditions, we found that primed treatments
354 tend to exhibit an increased root system convex hull allowing a greater range of nutrient
355 accessibility and therefore being more robust facing severe environmental conditions. This
356 improvement of root system architecture is a result of accelerated germination and therefore
357 improved growth during the first four days of growth but potentially still present throughout
358 the majority of the plant development with regards to the root area of exploration. The
359 understanding of root architectural changes facilitated by priming helps to improve the
360 selection of appropriate priming methods.

361 **6. Appendices**

362 Figure A1: Step by step procedure for transferring soil columns
363 Table A1: ANOVA table for all combinations of factors and contrasts
364 Table A2: ANOVA table for lateral root characteristics
365 Figure A2: Correlation of radicle growth and seed-soil contact

366 **7. Acknowledgements**

367 This work was financially supported by Syngenta. We acknowledge the help and support of
368 Stefan Mairhofer from the Hounsfield Facility for the development of the imaging tool to
369 measure root angle and convex hull. We acknowledge Murray Lark for his assistance in
370 developing an appropriate statistical model for the data analysis.

371 **8. Funding**

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373 **9. References**

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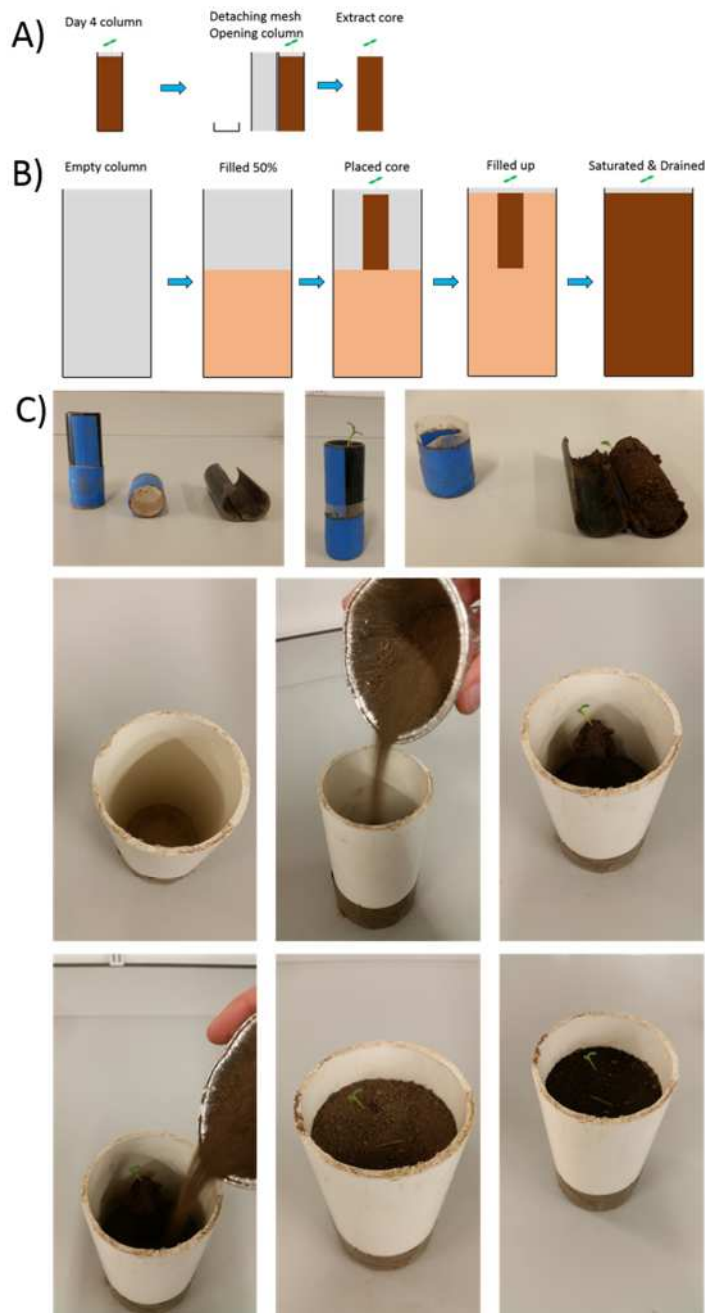
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461

462 Fig. A.1: Extraction and transplantation procedure of a small soil core into a larger
 463 polypropylene column. A) Extraction of a small soil core by detaching the mesh and opening
 464 the column. B) An empty column was filled with dry soil to approximately 50% of height and
 465 the soil core placed centrally on top. The column was filled with additional soil and saturated
 466 and drained after. C) Photography of the procedure step-wise.

467

468 Table A.1: ANOVA table for all analysed combinations of factors and contrasts.

	denDF	t-value	p-value
Batch	27	2.583932	0.0155
Treatment C1	27	1.325418	0.1961
Treatment C2	27	0.503395	0.6188
Priming	27	3.259726	0.0030
Day C1	56	20.836270	0.0000
Day C2	56	-3.571942	0.0007
Treatment C1 : Priming	27	-0.944208	0.3534
Treatment C2 : Priming	27	-0.243889	0.8092
Treatment C1 : Day C1	56	3.570404	0.0007
Treatment C2 : Day C1	56	3.892428	0.0003
Treatment C1 : Day C2	56	2.224063	0.0302
Treatment C2 : Day C2	56	3.911258	0.0003
Priming : Day C1	56	1.902555	0.0622
Priming : Day C2	56	-2.526829	0.0144
Treatment C1: Priming : Day C1	56	-2.595295	0.0120
Treatment C2: Priming : Day C1	56	-2.736383	0.0083
Treatment C1: Priming : Day C2	56	-1.547471	0.1274
Treatment C2: Priming : Day C2	56	-2.932048	0.0049

469

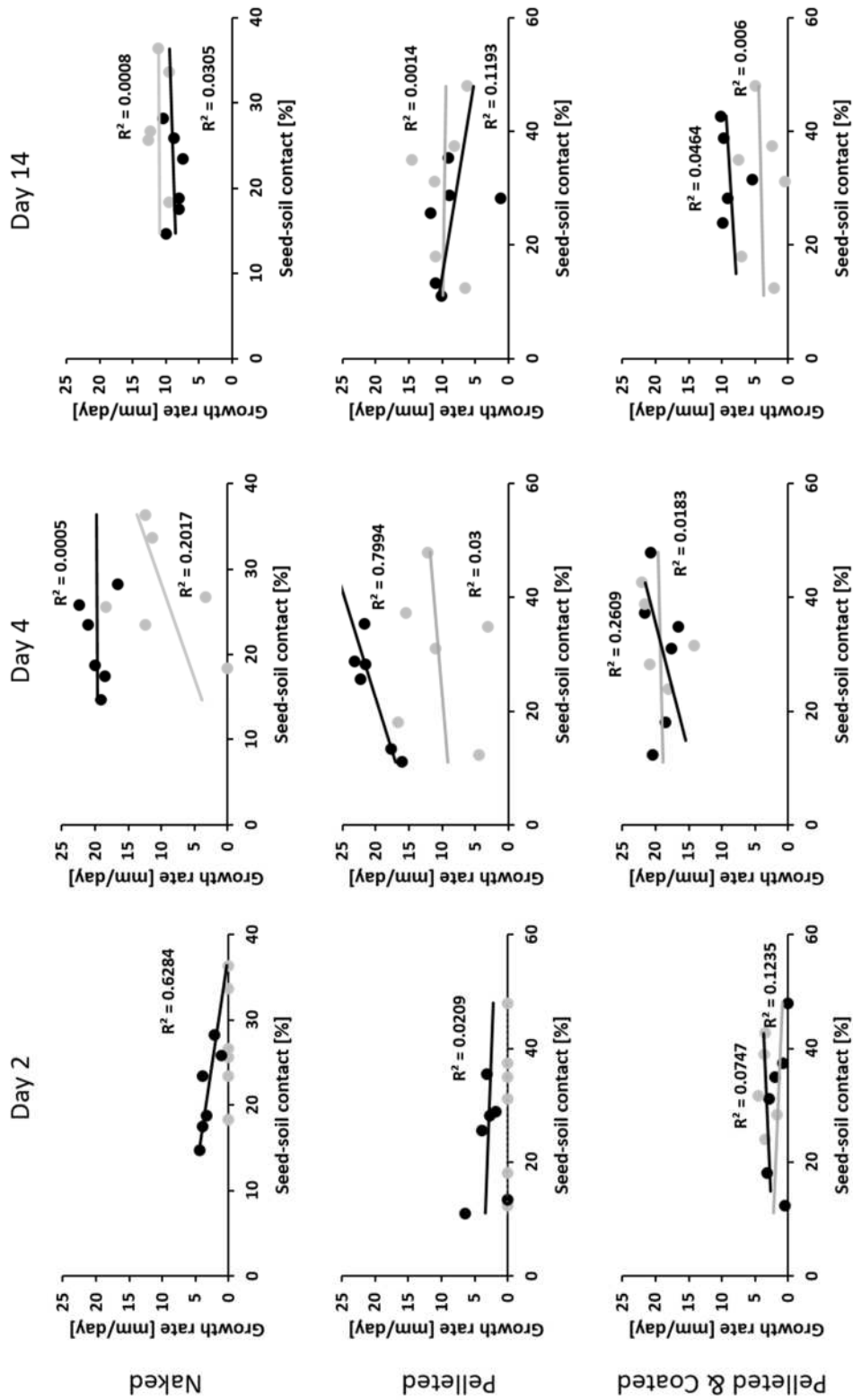
470 Table A.2: ANOVA table for lateral growth characteristics in all combinations of factors and
 471 contrasts.

	Number of laterals [#]			Total lateral length [mm]			Average lateral length [mm]		
	denDF	t-value	p-value	denDF	t-value	p-value	denDF	t-value	p-value
Batch	27	2.4553	0.0208	27	3.3917	0.0022	27	2.6528	0.0132
Treatment C1	27	-3.8665	0.0006	27	-3.5022	0.0016	27	-2.7415	0.0107
Treatment C2	27	-1.5348	0.1365	27	-2.5793	0.0157	27	-1.7100	0.0987
Priming	27	1.6933	0.1019	27	1.9108	0.0667	27	1.7502	0.0914
Treatment C1:Priming	27	1.5535	0.1319	27	1.3548	0.1867	27	1.4595	0.1560
Treatment C2:Priming	27	1.7943	0.0840	27	3.1908	0.0036	27	2.3794	0.0247

472

473

474 Fig. A.2: Correlation of seed-soil contact percentage and root length growth rate per day. The
475 root length growth rate was calculated as the difference in root length of the current day and
476 the previous measurement day divided by the number of days of growth. Seed-soil contact



477 determined at day 2 and used for correlation for root lengths at all time points. Grey indicates
478 a non-primed seed; Black indicates a primed seed.

479

480