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

Sebastian Blunk[1], Martine I de Heer[2], Ali Hafeez Malik[3], Kenneth Fredlund[3], Tobias Ekblad[3], Craig J Sturrock[1], Sacha J Mooney[1]

[1] Division of Agriculture and Environmental Science – University of Nottingham, Nottingham, UK

[2] Syngenta Ltd, Bracknell, UK

[3] Syngenta Seeds AB, now: Maribo Hilleshög Research AB, Landskrona, Sweden

Corresponding author: Sebastian Blunk – sebastian.blunk@nottingham.ac.uk

Abstract

Introduction

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

Results

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

Conclusions

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

Keywords

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

Abbreviations

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

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

1. Introduction

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

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

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

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

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

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

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

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

2. Materials & Methods

2.1. Treatment preparation

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

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

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

2.2. Soil core transplantation

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

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

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

2.3. Image analysis

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

2.4. Statistical analysis

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

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

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

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

3. Results

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

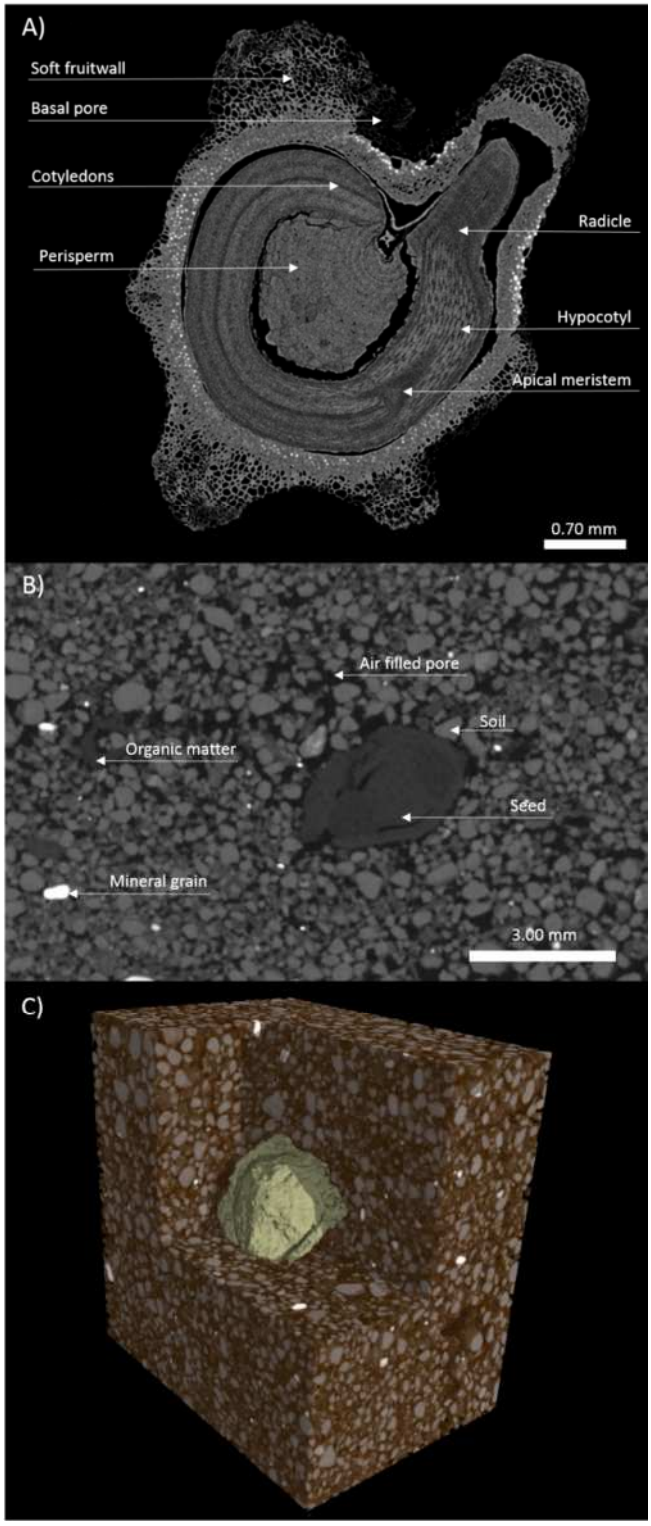

Figure 1: Quantification of a naked untreated seed. A) 2D X-ray CT image of a bare seed outside of soil. B) 2D X-ray CT image of a seed within the soil matrix surrounded by aggregates, air and water filled pores, organic matter and mineral grains. C) 3D rendered X-ray CT image of a naked seed in soil.

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

Table 1: Quantification of seed embryo and perisperm volume and surface area. Errors were 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)

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

Table 2: ANOVA table of factorial analysis including orthogonal contrasts for factors treatment and day. numDF = degrees of freedom in the numerator; denDF = degrees of freedom in the denominator; F-value = ratio of variance of group means and the mean of the variances within 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

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

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

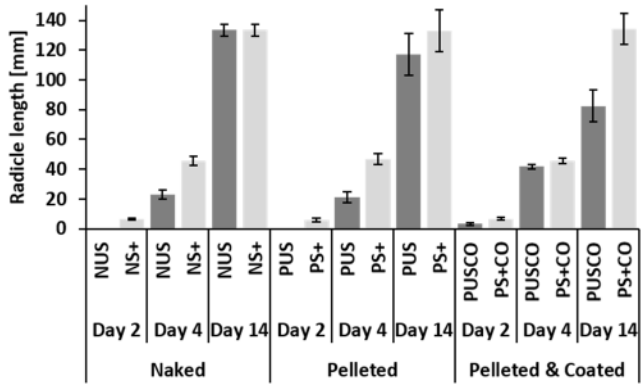

Figure 2: Root growth of sugar beet seedlings over time showing three treatment comparisons as pairs (dark: non-primed; light: primed). Error bars calculated as a standard error of the mean.

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

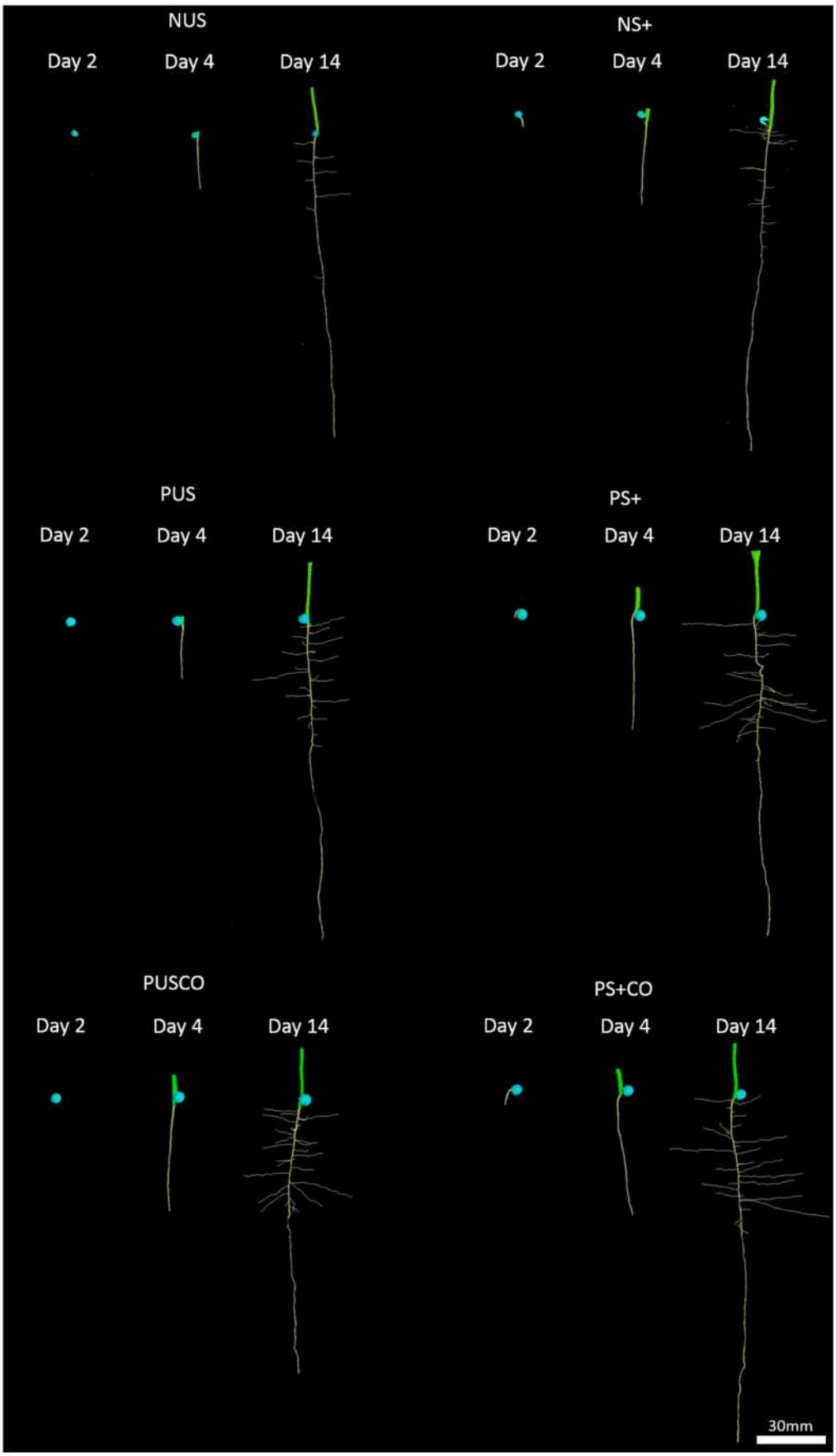

Figure 3: Temporal growth representation of one representative seedling per treatment.

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

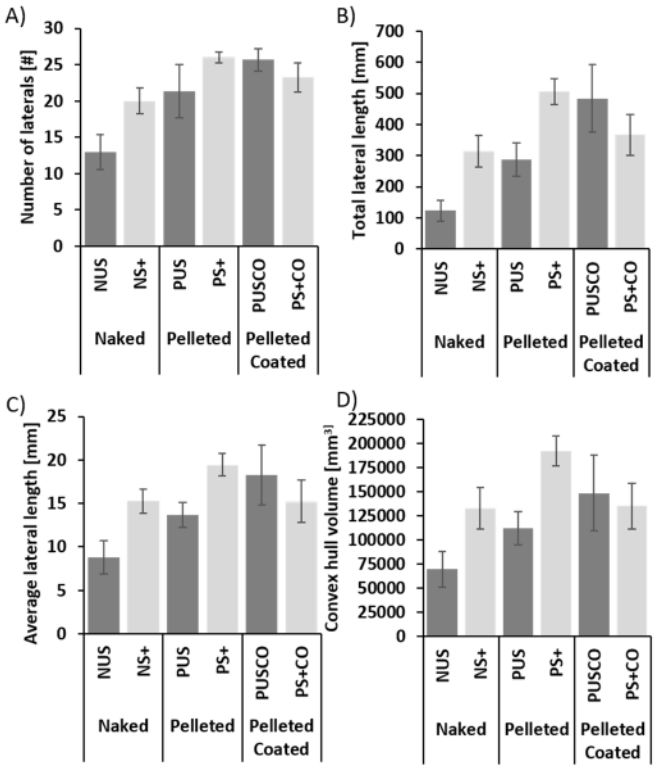

Figure 4: Growth comparison of lateral roots after 14 days of growth. A) Number of laterals counted. B) Total lateral length calculated as accumulative length of all laterals. C) Average length calculated as a ratio of total lateral length divided by number of laterals. Error bars calculated as standard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

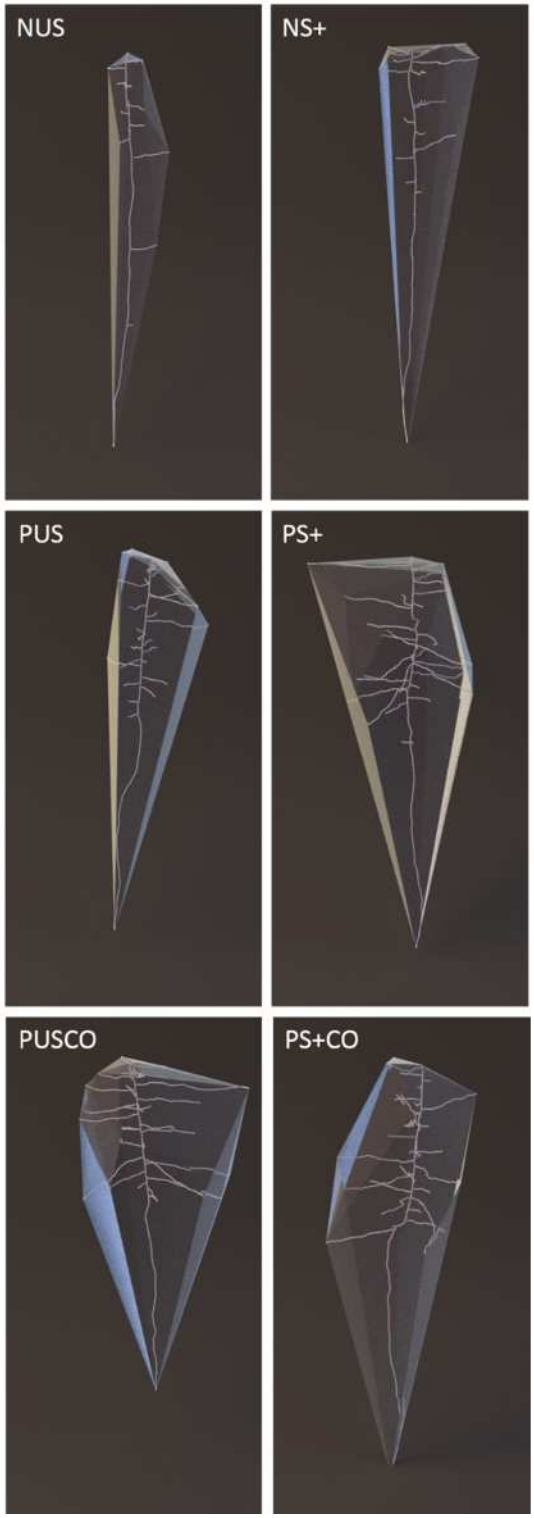

Figure 5: Convex hull of a representative root system from each treatment presented in Figure 4. The smallest convex object set surrounding 100% of the length of all lateral roots and the primary root.

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

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

4. Discussion

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

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

5. Conclusions

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

6. Appendices

Figure A1: Step by step procedure for transferring soil columns

Table A1: ANOVA table for all combinations of factors and contrasts

Table A2: ANOVA table for lateral root characteristics

Figure A2: Correlation of radicle growth and seed-soil contact

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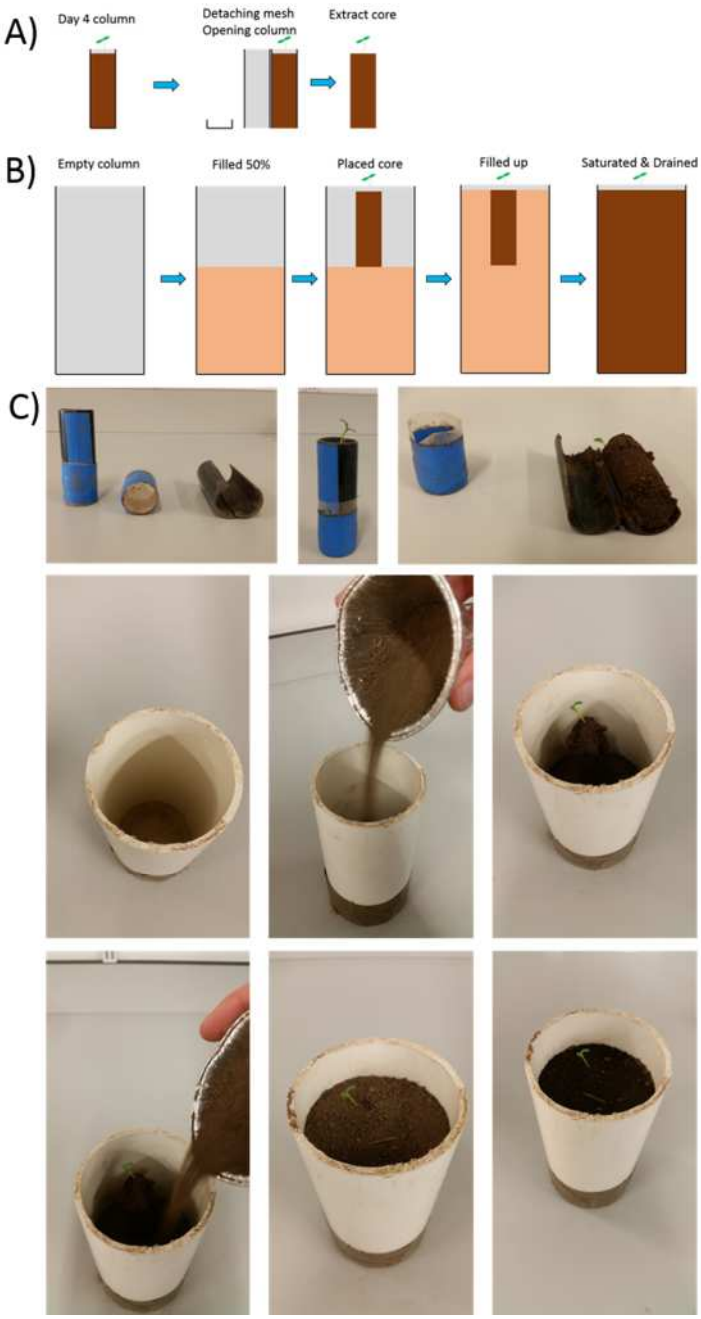

Fig. A.1: Extraction and transplantation procedure of a small soil core into a larger polypropylene column. A) Extraction of a small soil core by detaching the mesh and opening the column. B) An empty column was filled with dry soil to approximately 50% of height and the soil core placed centrally on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

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

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

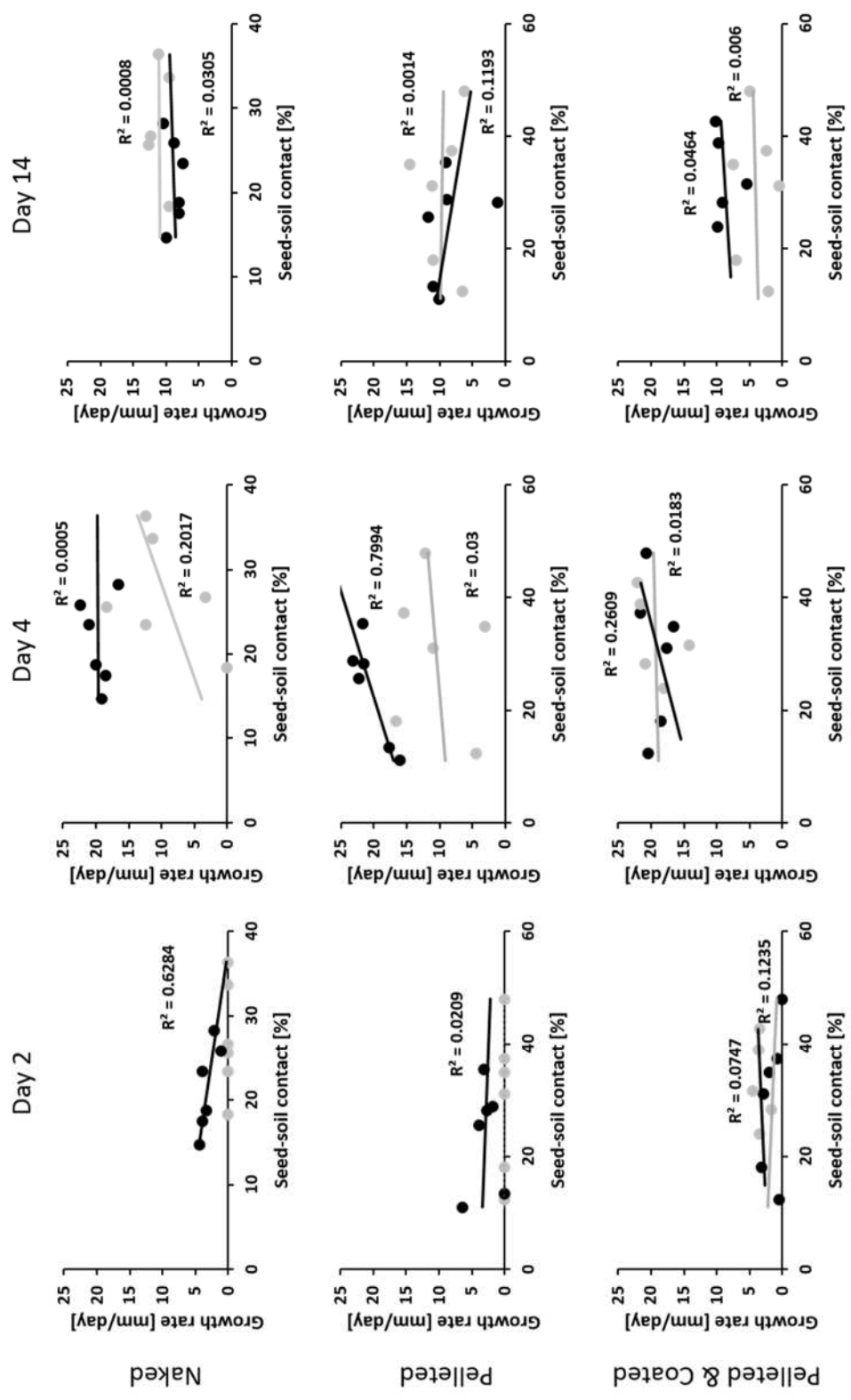

Fig. A.2: Correlation of seed-soil contact percentage and root length growth rate per day. The root length growth rate was calculated as the difference in root length of the current day and the previous measurement day divided by the number of days of growth. Seed-soil contact

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.

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