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# The Effect of Sugar Beet Seed Priming on Sugar Beet Yield and Weed Suppressive Ability

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**Abstract:** For optimal development in the field, sugar beets require fast emergence and rapid establishment of a homogenous stand. Environmental influences such as low soil temperatures or crusting of the soil surface usually slow down crop emergence and early development. Priming of the sugar beet seeds has proven to be a cost-effective method facilitating the rapid formation of a dense crop stand. Market penetration of the seed priming technology is variable. It ranges from very high in Western Europe and the USA to minimal in Eastern Europe. In this study, one commercial activated sugar beet variety was analysed under controlled climatic conditions in the growth chamber, in the greenhouse and in a field environment. Under controlled conditions in petri-dishes and in the greenhouse, seed priming significantly accelerated seed germination and reduced the time until the maximum number of sugar beet plants had emerged from 12 days to 6 days after seeding. In the field however, no significant effect of seed priming on sugar beet emergence was observed. Weed density, weed biomass and relative weed cover were similar in the activated and non-activated seed treatments indicating that seed priming did not increase competitive ability of sugar beets. Yields of both treatment were equal. Seed priming seems to be only beneficial under controlled and optimal growing conditions.

**Keywords:** Seed Treatment, Seed Activation, Germination Test, Weed Competition

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## 1. Introduction

Sugar beet (*Beta vulgaris* L.) is playing an important role in world food chain, supplying about 20% of world refined white sugar [8]. The number and variety of climates and soils, where the sugar beet is cultivated is very wide. Like other row crops, sugar beets are most sensitive to weed competition during the initial phase of crop development. During this critical period of sugar beet development the crop responds to weed competition with yield reduction [11, 14, 21]. The duration of the critical period depends on the time of the emergence of crop and the speed of early development. Therefore, it must be one objective in sugar beet cultivation to minimize the duration of this period during which the crop appears most vulnerable.

Seed priming seems to be a cost-effective and simple tool reducing the time needed for sugar beets to emerge and secure good yield [14, 16, 22, 26, 27, 30, 33]. The share of

primed seeds on the key markets, like the USA, France, Germany or the UK, is high. However, in Eastern-European countries seed priming technology plays only a minor in sugar beet seed market.

The priming process exposes the seed in a controlled way to a limited amount of water. The purpose of priming is to trigger the initial steps of germination. However, the initiation of germination must not go beyond the point, where process becomes irreversible since primed seeds still need to be further processed, transported and stored until seeded. In order to avoid the difficulty to manage short time of exposure to liquid pure water, which bears the risk of seed hydration beyond the point, where the germination process can be temporarily stopped, more sophisticated methods have been developed. The main seed priming methods include: hydro priming, solid matrix priming and osmo-priming. During simple hydro priming process, the seeds are exposed to the aqueous system under strict time control (steeping) or to the limited amount of water uptake (drum priming) [10]. In case

of osmo-priming, the limited seed hydration is achieved by putting the seeds into aerated solution of low water potential, containing inorganic salts, mannitol or polyethylene glycol [4, 12, 35]. The method of solid matrix priming involves placing the seeds into aqueous solution together with a certain amount of an absorbent – solid particles. The ratio between these three components determines the seed hydration [9, 16]. While hydro priming requires strict time control to avoid over-hydration, in osmo-priming and solid matrix priming the availability of water is limited by the liquid or solid components of the chemical environment in which priming takes place. Therefore, water exposure time control requirement is less strict and the risk of over-hydration is largely reduced.

Rapid emergence and early canopy closure might reduce weed competition in sugar beet and shorten the critical period of weed control, both leading to less yield losses and lower costs for weed control [6, 19].

The purpose of this study was to test the effect of sugar beet seed priming on the time of seed germination, crop emergence, early establishment and yield. It was further investigated if activated sugar beets were more competitive against weeds. The experimental questions are:

1. Do primed sugar beets germinate and emerge faster than non-primed plants?
2. Do primed sugar beets yield higher, have more sugar content and produce more white sugar than non-primed sugar beets?
3. Does priming provide better weed suppression?
4. Are effects observed under controlled conditions in the climate chamber and the greenhouse reproducible under the more variable environmental situation in the field?

## 2. Material and Methods

### 2.1. Sugar Beet Seeds

Primed and non-primed seeds of the same genotype were provided by Germains Seed. The priming method itself remained undisclosed.

### 2.2. Petri Dish Studies

To compare activated and not activated seeds, a series of two independent germination tests in petri dishes was first conducted. The layout was in randomized complete block design with four replications. The tests were carried out in 9 cm Petri dishes. Total number of seeds in each treatment was 100, 25 per petri dish. The seeds were placed on a filter paper disc, then moistened with distilled water at a ratio between seed weight and water of 1:2. To prevent dehydration by water evaporation, the dishes were hermetically closed. Petri dishes were placed in a climate chamber at 25°C and 16 hours light period. Germination progress was monitored daily. Germinated seeds were removed after germination and the dish was closed again. The seed was considered germinated after the emerged radicle overtopped the seed surface for at least two mm. To describe the results means of

two separate studies were calculated and expressed as percent germinated seeds. The uniformity of results is shown graphically by adding  $\pm$  standard deviation to the bars.

### 2.3. Greenhouse Pot Studies

Emergence and early development of primed and non-primed seeds was investigated in a set of greenhouse experiments. Two germination tests in a soil seedbed were carried out in plastic pots in the greenhouse. Seeds were planted into 15 cm x 15 cm x 15 cm pots. Seeds were placed 3 cm deep in accordance with conventional seeding practice. Soil mixture consisted of 60 parts loam, 30 parts compost soil and 10 parts sand. Daytime temperature during a 16-hour photoperiod was 25°C with 18°C at night. The pots were placed in a randomized complete block experimental design.

Percentage of emergence of the seedlings was measured after 6 days and then daily until 14 days after sowing. Fresh biomass of the plant shoot and leaf area of the plants were measured every day after emergence. For the leaf area measurement an RGB image of an area of 10 cm<sup>2</sup> in cotyledon stage of the plants and of 625 cm<sup>2</sup> at later stages was taken digital RGB camera Canon 500d. The plant images were further processed by binary black and white conversion with “ImageJ”, version 1.47a Green leaf parts were converted into white pixels and all other areas of the black pixels as final output the software provided the percentage of white pixels. On this numeric basis the leaf coverage area was in cm<sup>2</sup>. After the third pair of true leaves had unfolded the study was stopped because the pot size limited the further crop growth. 17 days after emergence when the samples of fresh biomass were analysed and dried in the drying chamber the proportion of dry biomass in total shoot weight was calculated. Percentage of the dry matter of the primed and not primed sugar beets was calculated at the time of the final measurement.

### 2.4. Field Experiments

In 2013 and 2014, two field studies were carried out at the field research station of the University of Hohenheim, Ihinger Hof in The Federal state of Baden Württemberg, Germany (48°74’N, 8°92’O, 478 m altitude). The region, where the field experiment was conducted is representative to the typical sugar beet cultivation zone. The experiments were set up as a randomized complete block design with four repetitions. Plots had a size of 3 m x 12 m.

The soil at the experimental field was a Haplic Luvisol with clay loam soil texture in the topsoil [5]. This soil has good water retention capacity and high fertility [3]. The climate at Ihinger Hof station offers sufficient rainfall during sugar beet growing period with long-term average rainfall of 691 mm and average temperature of 8.3°C. Weather data of both years are given in Table A1.

It was counted how many sugar beet seedlings of the primed and non-primed seeds had emerged and crop leaf area and biomass was measured. Assessments were made daily until 17 days after sowing in a frame of 0.25 m<sup>2</sup> per plot. The

dynamics of crop development before emergence were assessed by digging the soil in the randomly selected area within the experimental plots on an area of 0,1m<sup>2</sup> two times per plot. Weed density, weed biomass, crop biomass and weed cover was assessed at 8-leaf stage of sugar beet in a frame of 0.25 m<sup>2</sup> four times per plot. At time of harvest, sugar beets were remove from the soil in a subplot of 2.5 m<sup>2</sup> in the centre of each plot. Beets were washed, weighed and analysed for the sugar beet content. Then the beets were sliced, and the white sugar yield was determined in the laboratory according to the standard procedure.

### 2.5. Statistical Analysis

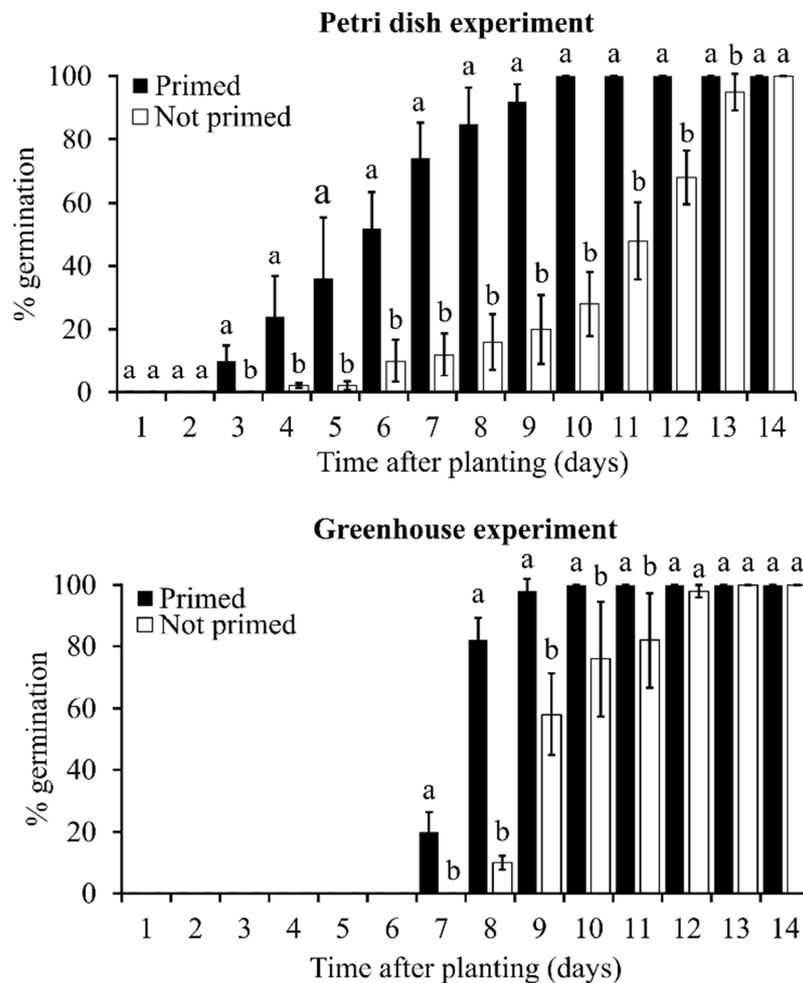
Statistical analysis of the experimental data was performed using statistical language R version 3.4.3 [29]. Shapiro-Wilk test was used to check the normality of the data. Laverne-test was used for checking the homogeneity of variance.

Comparison tests were conducted by means using the liner model (lm) followed by Tukey-Test at a significance level of  $\alpha \leq 0.05$ .

## 3. Results

### 3.1. Petri-Dish Study

Generally, seed priming has increased the speed of seed germination. Primed seeds started to germinate three days after the start seeding. Four days after seeding, 25% of the primed seeds had germinated compared to 2% of non-primed seeds. Primed seeds needed 6 days to reach 50% germination, compared to 11 days for the non-primed seeds. After 10 days, all primed seeds had germinated. The non-primed seeds needed 14 days for 100% germination (Figure 1).



**Figure 1.** Progress of seed germination/emergence in petri dish and greenhouse experiments. Bars represent mean of data from two separate studies (Closed bars represent activated sugar beets; open bars – not activated sugar beets)  $1 \pm$  Standard deviation. Means with different letters indicate significant differences between treatments (Tukey's test,  $P \leq 0.05$ ). Y-axis displayed in percent.

### 3.2. Greenhouse Study

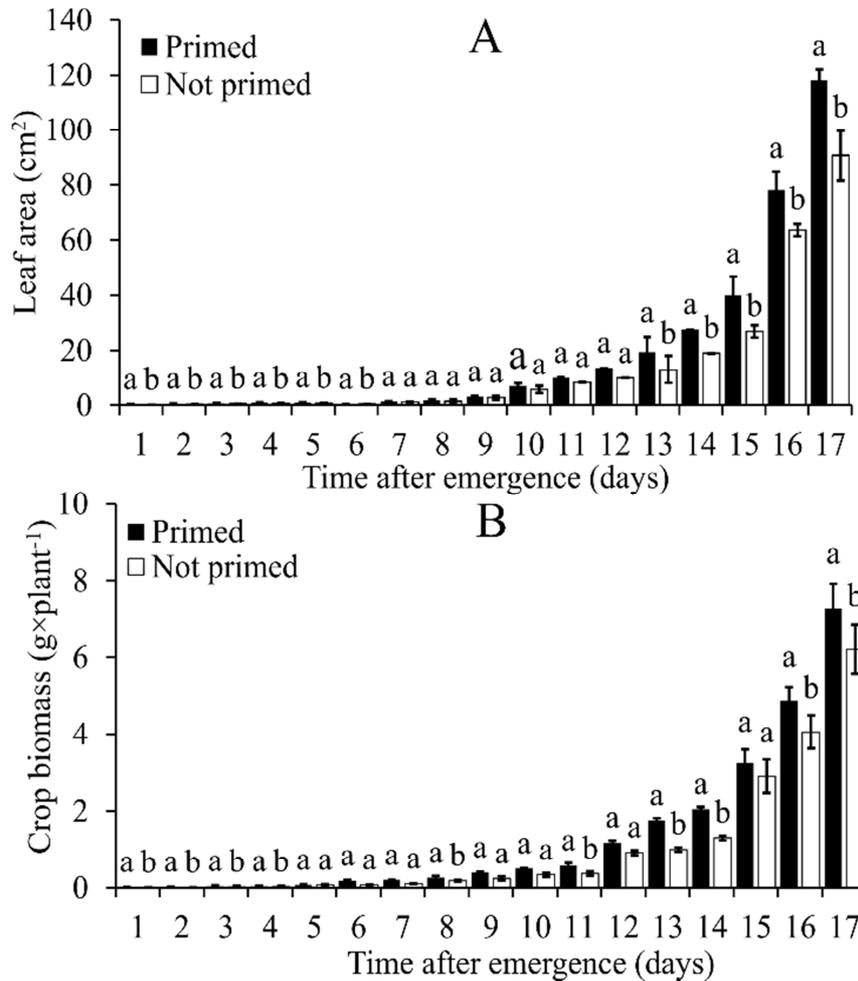
Emergence of sugar beet seeds in the pots was faster than germination in the petri-dishes. Similar, to the petri-dish study, primed seeds emerged faster than non-primed seeds.

Primed seeds needed 10 days for 100% emergence and non-primed seeds 13 days (Figure 1).

Due to rapid germination and emergence, plants from primed seeds have accumulated higher amounts of biomass compared to non-primed seeds. Starting from 13 days after

emergence, a significant difference between treatments was observed. The analysis of the crop canopy formation gave

similar results, showing higher leaf area of plants from primed seeds than of the non-primed seeds (Figures 2A and 2B).



**Figure 2.** A. Development of leaf area formation. Y-axis displayed in square centimetres. B. Development of above ground crop fresh biomass. Y-axis displayed in grams. Bars represent mean of data from two separate studies (Closed bars represent activated sugar beets; open bars – not activated sugar beets)  $1 \pm$  Standard deviation. Means with different letters indicate significant differences between treatments (Tukey's test,  $P \leq 0.05$ ).

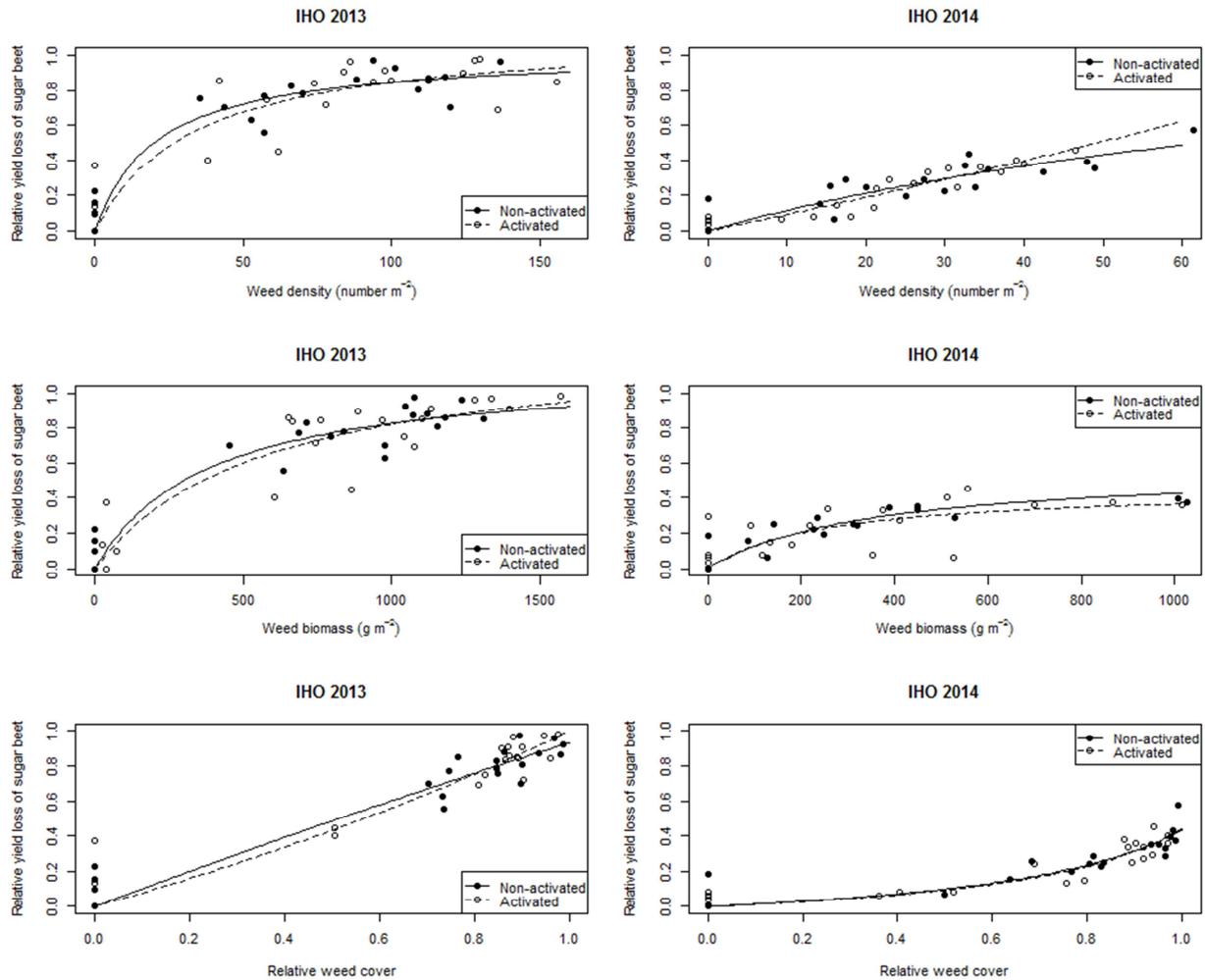
### 3.3. Field Study

In 2013, spring weather after planting was cold and wet with 45 mm rainfall with 14 days. Temperatures dropped to -0.7°C times for 9 days after planting. Therefore, sugar beets emerged later than in 2014. For the first 14 days after sowing in 2013, there were 8 rainy days with total precipitation amount with more than 45 mm. hours. In the 2014 cropping season, two frosty days with minimum air temperatures of -1.7°C were encountered 6 and 7 days after sowing. Soil temperature however, did not fall lower than 1.3°C even during frost times. Thus, in 2013 crop emergence took 14 days from the seeding date. The germination rate during two years of experiments was equal to 95 and 97% germination rate in cropping seasons of 2013 and 2014 respectively. The speed of crop establishment, described in BBCH phenological stages also differed between crop season of 2013 and 2014 (see Table A2). Hence, during the cropping seasons of 2013 and 2014 primed seeds have emerged with the same speed as non-primed seeds and leaf area was similar of primed and non-primed treatment (Table A3). In 2014,

priming treatment has shown some superiority over non-primed seeds, however this difference was statistically not significant.

During the season of 2014, due to the favourable climatic conditions, crop emergence, establishment and further development was faster compared to the 2013. Low temperatures in April did not cause any damage to sugar beets. A favourable amount of precipitation may have contributed to the normal crop establishment.

Weed infestation in 2013 was higher than in 2014. In 2013, weed density reached a maximum of 150 plants m<sup>-2</sup> with *Matricaria inodora*, *Chenopodium album* and *Polygonum lapatifolium* being the dominant species. Weeds caused yield losses of almost 80% in the untreated control compared to a standard herbicide treatment. In 2014, weed infestation rates were approximately 50% lower than in 2013. Thus, a maximum yield loss of 45% was calculated. Weed suppressive ability of sugar beet was very low. Primed seeds did not achieve a higher weed suppressive ability compared to non-primed seeds.



**Figure 3.** Response of activated and non-activated sugar beets in the field experiments in 2013 and 2014 to weed competition; regression model of Cousens (1985) [36] was used to calculate yield loss functions.

Sugar beet yield, sugar content and white sugar yield were not affected by seed priming in both years (see Table A4). As a consequence of the rough early season weather in 2013 the beet yield was reduced compared the yield in 2014.

## 4. Discussion

The effect of priming on sugar beet germination and emergence was significant in petri dishes and plastic pots. This corresponds to the study of Adel et al. (2017) which stated that pre-sowing seed priming treatments initiate metabolic processes [1]. Drying back seeds does not reverse this process [15]. This is well in line with our findings, where primed seeds demonstrate advantages over not primed seeds in speed of germination, emergence and further development under controlled environmental conditions.

Murray and Swensen, 1993, have conducted a study including petri dish test to obtain seed germination percentage and study of plant emergence under stress conditions such as compacted sand constant low temperature of 10°C [2]. These studies were conducted in the growth chamber and they didn't show significant advantage of the primed seeds over not

primed. This result does not correspond with present studies in the controlled environment, however it complies with the results of the field studies. The greenhouse studies show that under optimal environmental conditions, seed priming enhanced seed germination and the succeeding accumulation of crop biomass. The crop canopy area was also significantly higher for plants from primed seeds at the BBCH 14 stage of phenological plant development. The speed of growths of the crop canopy plays an extremely important role in sugar beet development under competition pressure from weeds [11]. It is decisive on the relative amount of interception of photosynthetically-active radiation by the weeds and the crop and thus impacts energy fixation and sucrose accumulation [18, 31, 34]. O'Donovan et al. (1985) have concluded that the time of weed emergence relative to crop emergence is also influencing the yield loss of the crop to competing weeds [25]. Sugar beet is especially sensitive to weed competition during the critical period in the early stages of crop development [20]. Thus, accelerated crop emergence establishment to a homogenous crop stand, leading to rapid canopy formation contributes to enhanced crop competitiveness against weeds. As a result, seed priming can be used as a tool that facilitates

integrated weed management [19].

Advancements in area of seed technology - alternative types of seed coat pellet, consisting of wood fibre with better porosity than a clay material resulted in faster germination [7, 25, 32].

Variation of the sowing depth and thus the cut of the distance that the seedling needs to cross before it gets to the soil surface, may strongly shorten the time of sugar beet emergence. In some regions of the Eastern Europe on lighter soils in arid environmental conditions effect of rolling is not sufficient to supply adequate germination and crop stand. Therefore, it is a common practice to sow the seeds deeper to prevent desiccation of the seedling [28].

Based on the promising results of climate chamber and greenhouse experiments, the effect of seed priming was analysed in field conditions in open environment. Analogically to the field study of Rykbost and Dovel (1997), our own studies have not shown any significant differences between primed and not primed sugar beets in speed of emergence, crop density, canopy formation, weed suppressive ability and the white sugar yield [32]. Authors assume that early sowing in colder soil would increase the difference between treatments. A study of Khan *et al.* (1983) has shown faster germination of PET (polyethylene glycol) osmo-primed table beet seeds in cold soils [17]. However, in contrast to that study, Murray and Swensen (1993) have shown no significant differences in seed germination between different temperature regimes [23].

In the present study the adverse climate conditions during cropping season 2013 may have influenced seed germination. The high amount of rainfall may have reduced the oxygen supply to the seed, and slow down or inhibit the germination by limiting respiration intensity. Two weeks after sowing, soil temperatures had average values of 8.6°C and 12°C in 2013 and 2014 respectively. These temperatures are assumed to cause no damage to sugar beet, as reported in the study of Capron *et al.* (2007). Short drops of the ambient air temperatures didn't affect seeds sown into the soil with high clay content.

The productivity of the sugar beets measured as total harvested weight, sugar content and white sugar yield was not affected by the priming treatment. Priming of the seeds

has reduced the variation of sugar beet yield, sugar content and white sugar yield between replications of the study in 2014, similarly to the study of Finch-Savage and Bassel (2015) [9]. In 2013, priming did not have any effect on any yield parameter.

The experiments have clearly demonstrated that priming positively impacted the early development of sugar beets. Under highly controlled condition established in a climate chamber or a greenhouse seed germination, plant emergence, early formation of crop canopy, and biomass accumulation were significantly enhanced. The clear reproduction of these findings under field conditions was not possible. Though some isolated findings pointed into the same direction like the results obtained indoors. Failure of reproduction may have been caused by possible and compensatory interferences within the multiplicity of factors encountered and may have offset the limiting impact of one or few individual factors. Possibly also the conditions encountered at the site of the field studies may not have been limiting enough to trigger the evident appearance of advantages caused by the priming treatment the tested sugar beet seeds were subjected to.

## 5. Conclusions

Priming of sugar beet seeds speeds up sugar beet germination and emergence. However, less favourable growing condition as it often occurs in the field may overcome the positive effects of seed priming. Therefore, more investigations are needed to test seed activation under field conditions.

## Acknowledgements

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## Conflict of Interest

All the authors do not have any possible conflicts of interest.

## Appendix

*Table A1. Climate data during the two experimental seasons.*

Parameter	Year	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.
Sum of monthly precipitation, mm	2013	45.4	138.6	82.8	173.4	69.5	95.9	—
	2014	41.4	68.2	24.3	162.0	142.4	77.0	6.3
Air temperature, month average, °C	2013	8.4	10.8	15.8	19.8	17.6	13.7	—
	2014	10.9	12.1	16.7	18.4	15.7	14.6	12.1
Average soil temperature, °C	2013	8.6	12.1	17.9	22.2	19.3	15.0	—
	2014	12.1	14.2	19.9	19.2	17.2	16.0	12.3
Minimal air temperature, °C	2013	-4.2	0.5	7.2	7.5	7.7	5.7	—
	2014	-1.7	1.2	3.9	8.4	6.9	3.3	1.3
Minimal soil temperature, °C	2013	-0.7	4.9	9.0	10.5	11.2	8.2	—
	2014	1.3	3.9	7.9	10.8	8.9	6.4	4.4

**Table A2.** Time (days) of crop establishment during field experiments; different letters indicate that treatments were significantly different (Tukey's test,  $P \leq 0.05$ ).

Year	Treatments	Time to reach phenological stages (days after planting)						
		BBCH 05	BBCH 10	BBCH 12	BBCH 14	BBCH 16	BBCH 18	BBCH 20
2013	Primed	11 <sup>a</sup>	22 <sup>a</sup>	33 <sup>a</sup>	44 <sup>a</sup>	56 <sup>a</sup>	67 <sup>a</sup>	74 <sup>a</sup>
	Non-primed	11 <sup>a</sup>	22 <sup>a</sup>	33 <sup>a</sup>	44 <sup>a</sup>	56 <sup>a</sup>	67 <sup>a</sup>	74 <sup>a</sup>
2014	Primed	8 <sup>a</sup>	11 <sup>a</sup>	26 <sup>a</sup>	38 <sup>a</sup>	46 <sup>a</sup>	54 <sup>a</sup>	63 <sup>a</sup>
	Non-primed	8 <sup>a</sup>	11 <sup>a</sup>	28 <sup>a</sup>	40 <sup>a</sup>	48 <sup>a</sup>	55 <sup>a</sup>	63 <sup>a</sup>

**Table A3.** Time of crop establishment during field experiment. Described by means of leaf area of one single plant; different letters indicate that treatments were significantly different (Tukey's test,  $P \leq 0.05$ ).

Year	Leaf area of one plant, plant $\times$ cm <sup>2</sup>					
	2 weeks after planting		4 weeks after planting		6 weeks after planting	
	Primed	Non-primed	Primed	Non-primed	Primed	Non-primed
2013	0	0	11.5 a	12.1 a	112.3 a	115.5 a
2014	5.9 a	5.6 a	138.1 a	124.7 a	177.5 a	175.3 a

**Table A4.** Basic data about field experiment and average sugar beet yield (t ha<sup>-1</sup>) and white sugar yield (t ha<sup>-1</sup>) of the weed-free control at two experimental locations. Small letters indicate significant difference between treatments. Multiple comparison test after Tukey at significance level  $p \leq 0.05$ .

Treatment	2013		2014	
	Activated	Non-activated	Activated	Non-activated
Planting date	18.04.2013	18.04.2013	11.04.2013	11.04.2013
Harvesting date	20.09.2013	20.09.2013	06.10.2014	06.10.2014
Vegetation period (days)	156	156	178	178
Seed use (number ha <sup>-1</sup> )	110,000	110,000	110,000	110,000
Crop density (plants ha <sup>-1</sup> )	105,000	105,000	107,000	107,000
Germination percentage (%)	95.5	95.5	97.2	97.2
Yield weed-free (t ha <sup>-1</sup> ±standard deviation)	78.3 <sup>a</sup> ±5.2	82.9 <sup>a</sup> ±5.9	91.3 <sup>a</sup> ±6.8	90.0 <sup>a</sup> ±10.8
Sugar content (%±standard deviation)	13.5 <sup>a</sup> ±1.6	15.4 <sup>a</sup> ±1.5	16.1 <sup>a</sup> ±0.7	15.7 <sup>a</sup> ±1.2
White sugar yield (t ha <sup>-1</sup> ±standard deviation)	10.6 <sup>a</sup> ±2.5	12.8 <sup>a</sup> ±2.5	14.3 <sup>a</sup> ±1.4	14.1 <sup>a</sup> ±1.6

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