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Hydro-thermal priming enhance seed germination capacity and seedling growth in sugar beet

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Abstract: Seed priming improves seed performance in many crop species. In this study, the influence of hydrothermal priming on seed parameters of sugar beet is investigated in both laboratory and field conditions. In the laboratory, the treatments consist of a combination of cultivars (Arya and Shokoofa), hydro-priming at two temperatures (10 and 15 °C) for 6, 10, 14, 18, 22 hours. Germination traits and seedling growth were measured for determination of optimum hydro-thermal priming. Also, the protein pattern in the optimum hydro-thermal priming treatments and unprimed seeds were compared by electrophoresis. In the field experiment, the percentage and rate of emergence of primed and unprimed seeds were measured. Results showed that hydro-thermal priming had a positive effect on final germination percentage, mean germination time and uniformity of germination. Optimum hydro-thermal priming time and the temperature were 6 and 10 hours at 15 °C for Shokoofa and Arya cultivars respectively. Hydro-thermal priming increased the seed emergence percentage in the field by 15%. There was no significant difference in protein pattern between primed and unprimed seeds. In general, hydro-thermal priming not only increases sugar beet seed germination in the laboratory but also has a more positive effect on the emergence in the field condition.

Key words: Seed emergence; Seedling vigor; Seed priming; Protein pattern.

Introduction

For sugar beet (*Beta vulgaris* L.) crop, uniformity of field emergence, in addition to emergence percentage, is crucial for final yield and quality. Therefore, the seed industry applies various pre-sowing treatments to enhance seed quality, such as priming, conditioning and coating (1). Also, sugar beet seed coats contain substances like phenols, ammonia, fat, oxalic acid, potassium nitrate, betaine and mucilage, whose negative effect can be eliminated by washing with water (2).

Seed priming (hydration) has successfully been applied to improve germination capacity and uniformity of seedling emergence of many crops (3, 4). However, the reaction to priming treatment seems to be different among crop species (5, 6). Understanding germination responses to priming based on the establishment of optimal treatment would be very important for the seed industry.

The uptake of water by seed occurs in three phases. There is a rapid initial water uptake (imbibition; phase I). In phase (II) or plateau phase, there is a less increase in water uptake, but germination physiology activities are initiated, including synthesis of nucleic acids (DNA and mRNA), enhancement of the number of mitochondria, protein and ATP production. In phase (III), there is a second increase in water uptake associated with the completion of germination and radicle emergence (7, 8). For avoid the onset of phase III during priming, water uptake must be stopped before germination is completed.

Seed priming is a method of hydration that necessary metabolic activities occurred for germination but radicle emergence is prohibited (5). It has been known that washing sugar beet seeds improve their germination and seedling establishment (9). These positive effects can be due to a decrease in the number of inhibitors from the pericarp and direct influence on the cell cycle in the embryo (10, 11). Water uptake in the seed of sugar beet leads to opening of the semi pericarp and the reduction of existing physical pressure, as well as the easier protrusion of radicle during germination, which increases seedling growth rate and ultimately increases root and sugar yield per unit area (12).

There is a debate about cell cycle activity during seed priming. Some researchers have reported that primed seeds increase their synthesis of DNA and RNA (13, 14), whereas some others reported differences in the reaction to the priming treatment for various species and seed lots (15, 16).

Electrophoresis (SDS-PAGE) is a practical and reliable method to detect the seed protein variations of crop germplasm (11). This method can be used to distinguish cultivars of particular crop species (17, 18). Kakaei et al. (19) examined the variation of rapeseed (*Brassica napus*) protein patterns by electrophoresis and reported that this method can be used as a simple and economical method for identification of genetic

diversity of germplasm. Bushehri et al. (20) observed that SDS-PAGE is a useful tool for characterization of soybean (*Glycine max* L.) cultivars by banding of seed protein pattern.

The purpose of this study was to determine the optimum level of hydro-thermal priming for seeds of two sugar beet cultivars and its effects on germination traits, seedling vigor and seed protein pattern.

Materials and Methods

Hydro-thermal priming experiment

The experiment was conducted in the seed laboratory of Kermanshah Agricultural and Natural Resources Research and Education Center (KANRREC), Iran. To determine the appropriate level of temperature and duration of hydro-priming, seeds of two sugar beet cultivars (Arya and Shokoofa) were received from Sugar Beet Seed Institute (SBSI), Iran. The commercial seeds had already been treated with carboxinthirum fungicide to protect fungal diseases. Hydro-priming was carried out with a full immersion of seeds in distilled water with the ratio of 1:5 (21), at two different temperatures of 10 and 15 °C (22, 23) for 6, 10, 14, 18 and 22-h inside a germinator (Model: Grouc 550G) accompanied with aeration. The experiment consisted of twenty priming treatments as well as two control treatments including unprimed seeds of the two sugar beet cultivars. During the hydrothermal priming period, aeration was performed with an aquarium pump to supply oxygen for seeds (24). The seeds were then taken out from water and dried up to original moisture (10 to 12% on a dry weight basis) at room temperature (20 °C) for approx. 24 h. Moisture of seeds was checked by electronic moisture meter (G-WON GMAK-503s). After priming 200 seeds of each treatment including primed and unprimed seeds of two cultivars were placed on pleated paper (PP) at four replications in the germinator adjusted on 25 °C \pm 1 for 8 days (25). Germination (protrusion of radicle by 2 mm) was measured in daily intervals and continued until fixed state. The number of germinated seeds was recorded as final germination percent (FGP) and the percentage of normal seedling was determined (26). Mean germination time (MGT) was calculated using the formula of Foti et al. (27):

Mean germination time (MGT) = Σ (N_iT_i/S)

Where N_i = number of germinated seeds at day i; T_i = day i; S = germinated seeds.

Germination Uniformity Index (GUI) for GUI 90-10 is time between 10-90% of G-max or germinated seeds (28).

Analysis of variance was performed based on a completely randomized design with four replications. Mean values were compared using LSD test (29).

Seedling growth test

The seedling growth rate (SGR) test, in which the weight of dried normal seedling axis is measured after germination, was conducted as described in the Vigor Testing Handbook (30) to estimate seed vigor. The primed and unprimed seeds from each cultivar (22 treatments), in four replicates of 50 seeds each, were placed in a row 8 cm from the top margin of moistened towel paper and rolled. Then the rolled papers placed in tubes

of 5 cm diameter and 17 cm in height and were placed in an upright position in a germinator at a constant 25 °C \pm 1 and 90% relative humidity. After 7 days, the normal seedlings (26) were counted and root and shoot length measured for each treatment. The seedling axes from each rolled towel paper were bulked, dried in a convection oven for 24-h at 80 °C and weighed.

The seedling growth ratio was calculated using formula (30) :

$$SGR = (RW + SW) / n$$

Where, SGR is seedling vigor ratio, RW is root dry weight (mg), SW is shoot dry weight (mg) and n is the total number of seedlings.

Protein quantification using SDS-PAGE

The protein pattern was evaluated by SDS-PAGE in the optimum hydro-thermal priming condition (10-h in Arya cultivar and 6-h in Shokoofa cultivar at 15 °C) as well as unprimed seeds of two sugar beet cultivars. To extract proteins for electrophoresis, seeds were ground to fine powder with mortar and pestle. Sample buffer $(400 \ \mu l)$ was added to 0.01g of seed flour as extraction liquid and mixed thoroughly in Eppendorf tube. The sample buffer used to load the samples in the different lanes contained 0.5 M Tris-HCl (pH 6.8), 2.5% SDS, 10% glycerol and 5% 2-mercaptoethanol. Bromophenol Blue (BPB) was added to the sample buffer as tracking dye to follow the movement of proteins in the gel. The protein extracts were analyzed by electrophoresis in the above sample buffer through the slab type SDS-PAGE using 11.25% polyacrylamide gel according to Laemmli (31). The gel was stained with 0.04% Coomassie brilliant blue for about 20-30 minutes and then destained in methanol, acetic acid and distilled water (45:10:45 v/v) until the color of background disappeared and the gels became clear (32). The gels were photographed to visualize the protein bands. To avoid confusion in the data analysis, the major protein bands between 14 kDa and 78 kDa were assayed for data recording.

Field experiment

The field experiment was carried out at KANRREC (Latitude 34° 16' N, Longitude 46° 50' E, Altitude 1365 m above sea level) in two years [2016-2017]. Soil texture was clay loam with EC of 0.68 dsm-1 and pH of 7.6. Percentage of emergence was investigated for optimum hydro-thermal priming condition (10-h in Arya cultivar and 6-h in Shokoofa cultivar at 15 °C) as well as unprimed seeds of two cultivars in the field condition. The experimental design was a factorial, based on RCB design with four replications. Each plot consisted of 4 rows with 2 m length and row space 50 cm. Seeds were hand sown in about 2-3 cm depth with a density of 100 seeds per plot in April 2016. Seedling emergence was recorded in daily intervals up to the day until maximum seedling establishment in each plot was achieved. Subsequently, the percentage and uniformity of emergence and mean germination time were calculated.

Analysis of variance of the data appropriate to the experimental design and comparison of means at $p \le 0.05$ and $p \le 0.01$ were carried out, using MSTATC software.

Results

Germination traits

Analysis of variance of the laboratory data showed that effects of temperature and duration of hydro-priming were significant for traits of final germination percentage (FGP) mean germination time (MGT) and germination uniformity index (GUI). The highest germination percentages were achieved for Shokoofa and Arya cultivars with 6 and 10 hours hydro-priming at 15 °C, respectively (Table 1). Lowest mean germination time (2.25 days) was obtained in case of seeds primed for 10-h at 15 °C in Shokoofa cultivar (Table 1).

The best germination uniformity index (which refers to the time between 10-90% of germinated seeds) was observed for Shokoofa and Arya cultivars under 10 and 14 h seed hydro- priming at 15 °C, respectively (Table 1).

Seedling growth traits

These results showed that traits of shoot and root length, root dry weight, root/shoot length, root/shoot dry weight and seedling growth rate (SGR) were significantly affected by temperature and hydro-priming duration. The root and shoot lengths increased in the primed seeds of the two sugar beet cultivars (Table 2). Different priming treatments had a different effect on seedling growth rate (SGR). According to this data, maximum seedling growth rate (SGR) was obtained for Arya cultivar with 22-hour hydro-priming at 15 °C (Table 2).

Field experiment

The effect of hydrothermal priming was significant

on final emergence percentage (FEP) in the first and second years. So that emergence percentage in primed seeds from 54.5% and 62% in control conditions in the first and second years increased to 70.8% and 73.8% respectively (Table 3). Interaction effect of hydro-thermal priming and cultivar were significant on final emergence percentage (FEP) in the first and second years (Table 3). The emergence percentage in primed seeds in Aria cultivar in the first year increased from 57.5% (control) to 65%, but in Shokoofa cultivar from 51.5% (control) to 76.5% respectively. The second year in Aria cultivar increased from 59% (control) to 80% but Shokoofa cultivar from 65% (control) to 67.5% respectively (Table 3).

The effects of hydrothermal priming and its interaction with cultivar were significant on emergence uniformity index (EUI) in first and second years. Primed seeds had greater uniformity of emergence than unprimed seeds in field condition, but significant differences between cultivars to given treatment were minimal (Table 4).

Correlation coefficients among the germination and emergence traits

A significant positive correlation (0.649^{**}) was observed between seed emergence percentage measured in the field and germination measured in the lab experiment. Furthermore, germination uniformity measured in the lab experiment showed a significant positive correlation (0.752^{**}) with emergence percentage measured in the field experiment (Table 5). There was a positive correlation (0.662^{**}) between emergence uniformity and percentage in the field experiment.

Table 1. Effect of hydrothermal priming treatments on the germination traits of two sugar beet cultivars.

	Priming	treatment				
Cultiver	Tomporature (°C)	Duration of hydro-	Final germination	Mean germination	Germination uniformity index	
Cultival	Temperature (C)	priming (hours)	Percentage	time (day)		
Arya	10	6	82.25 cd	3.66 а-с	3.52 a	
		10	89.50 a-d	2.52 а-с	2.02 b-d	
		14	86.50 a-d	3.02 а-с	2.10 b-d	
		18	87.50 a-d	3.54 а-с	3.14 ab	
		22	81.50 d	2.89 а-с	2.57 a-d	
Shokoofa	10	6	91.00 a-d	3.74 ab	3.14 ab	
		10	90.00 a-d	3.20 а-с	2.74 а-с	
		14	85.00 a-d	3.30 а-с	3.22 ab	
		18	90.00 a-d	3.32 а-с	2.62 а-с	
		22	82.35 cd	2.92 а-с	2.17 b-d	
Arya	15	6	91.50 a-c	2.82 а-с	2.07 b-d	
		10	96.00 a	2.38 bc	1.75 cd	
		14	91.50 a-c	2.35 bc	1.34 d	
		18	83.50 b-d	3.00 a-c	3.21ab	
		22	93.00 a-c	2.43 bc	1.69 cd	
Shokoofa	15	6	95.00 a	2.42 bc	1.87 cd	
		10	93.50 ab	2.25 c	1.34 d	
		14	92.50 a-d	2.65 а-с	2.37 a-d	
		18	87.50 a-d	3.34 а-с	2.89 а-с	
		22	88.50 a-d	2.86 а-с	2.10 b-d	
Arya (control)	-	-	82.95 cd	3.66 а-с	3.50 a	
Shokoofa (control)	-	-	85.50 a-d	3.96 a	2.83 а-с	

Mean values within a column with the same letter are not significantly different based at P < 0.05.

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Table 2:. Effect of hydrothermal priming treatments on the seedling growth traits of two sugar beet cultivars.

	Priming treat	ments						
Cultivar	Temperature (°C)	Duration of hydro-priming (hours)	Shoot length (cm)	Root length (cm)	Root dry weight (mg 25 plant ⁻¹)	Root/ shoot length	Root/ shoot dry weight	Seedling growth rate (mg seedling ¹)
Arya	10	6	4.51 ab	14.57 а-е	12.45 h	3.23 e-h	0.40 h	2.12 a-d
		10	4.00 c-f	15.63 а-с	15.30 e-h	3.92 а-с	0.53 d-f	1.92 cd
		14	4.61 ab	14.86 a-e	19.13 a-c	3.25 e-h	0.63 a-d	2.08 a-d
		18	3.65 f	16.20 a	17.30 a-f	4.44 a	0.57 c-f	2.12 a-d
		22	4.21 a-e	15.79 ab	17.50 a-f	4.16 ab	0.61 a-d	1.97 a-d
Shokoofa	10	6	3.97 c-f	14.89 a-e	14.93 f-h	3.85 b-d	0.55 d-f	1.86 d
		10	4.30 a-d	15.88 ab	16.05 d-g	3.69 b-f	0.59 c-f	1.94 a-d
		14	4.42 a-c	14.19 b-e	15.68 e-g	3.23 e-h	0.55 d-f	1.94 a-d
		18	4.55 ab	14.69 a-e	16.40 c-g	3.24 e-h	0.59 c-f	1.92 b-d
		22	4.31 a-d	15.81 ab	19.88 a	3.66 b-f	0.71 a	2.06 a-d
Arya	15	6	4.64 a	14.34 a-e	15.73 e-g	3.10 gh	0.51 e-g	2.17 а-с
•		10	4.54 ab	13.60 de	15.90 e-g	3.00 h	0.50 f-h	2.11 a-d
		14	3.96 c-f	15.15 a-d	18.83 a-d	3.86 b-d	0.60 b-e	2.18 ab
		18	4.41 a-c	15.02 a-d	14.38 cd	2.96 h	0.42 gh	2.14 a-d
		22	3.93 d-f	13.95 b-e	19.75 a	3.56 c-g	0.62 a-d	2.20 a
Shokoofa	15	6	4.50 ab	13.38 de	17.58 a-f	2.99 h	0.69 ab	2.04 a-d
		10	3.91 d-f	14.72 a-e	17.95 a-e	3.77 b-e	0.58 c-f	1.96 a-d
		14	3.97 c-f	14.66 a-e	17.05 a-g	3.72 b-f	0.55 d-f	2.12 a-d
		18	4.15 b-e	13.66 с-е	15.63 e-g	3.37 d-h	0.51 e-g	2.11 a-d
		22	4.02 c-f	14.66 a-e	16.98 b-g	3.66 b-f	0.54 d-f	2.09 a-d
Arya (control)	-	-	3.80 ef	13.02 c	16.93 b-g	3.60 c-g	0.65 a-c	1.92 cd
Shokoofa (control)	-	-	3.87 d-f	13.52 de	15.40 e-g	3.21 f-h	0.49 f-h	1.86 d

Mean values within a column with the same letter are not significantly different based at P < 0.05.

Table 3. Effect of priming treatment on the final emergence percentage of two sugar beet cultivars in the first and second years.

	Primi	ng treatment			
Cultivar	Temperature (°C)	Duration of hydro- priming (hours)	Final germination Percentage	Mean germination time (day)	Germination uniformity index
Arva	10	6	82.25 cd	3 66 a-c	3 52 a
<i>i</i> li yu	10	10	89 50 a-d	2 52 a-c	2.02 h-d
		10	86.50 a-d	2.52 a-c	2.02 b-d
		19	80.50 a-d	3.02 a-c	2.10 0-u 2.14 ab
		10	07.30 a-u	3.34 a-c	3.14 ab
	10	ZZ ć	81.30 d	2.89 a-c	2.37 a-d
Shokoofa	10	6	91.00 a-d	3.74 ab	3.14 ab
		10	90.00 a-d	3.20 a-c	2.74 a-c
		14	85.00 a-d	3.30 а-с	3.22 ab
		18	90.00 a-d	3.32 а-с	2.62 а-с
		22	82.35 cd	2.92 а-с	2.17 b-d
Arya	15	6	91.50 a-c	2.82 а-с	2.07 b-d
		10	96.00 a	2.38 bc	1.75 cd
		14	91.50 a-c	2.35 bc	1.34 d
		18	83.50 b-d	3.00 а-с	3.21ab
		22	93.00 a-c	2.43 bc	1.69 cd
Shokoofa	15	6	95.00 a	2.42 bc	1.87 cd
		10	93.50 ab	2.25 c	1.34 d
		14	92.50 a-d	2.65 а-с	2.37 a-d
		18	87.50 a-d	3.34 а-с	2.89 а-с
		22	88.50 a-d	2.86 а-с	2.10 b-d
Arya (control)	-	-	82.95 cd	3.66 а-с	3.50 a
Shokoofa (control)		-	85.50 a-d	3.96 a	2.83 а-с

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Table 4. Effect of priming treatment on the emergence uniformity index of two sugar beet cultivars in the first and second years.

Cultivars	First-year		Mean	Second-year		Mean
Treatment	Arya	Shokoofa		Arya	Shokoofa	
Hydro-thermal priming	3.6 b	3.4 b	3.5 b	3.4 b	3.8 b	3.6 b
Control (untreated)	4.9 a	4.7a	4.8 a	4.8 a	4.2 a	4.5 a
Mean	4.2 a	4.1a		4.1 a	4 a	

Mean values within a column with the same letter are not significantly different based at $P \le 0.05$.

Table 5. Correlation coefficients between evaluated traits in sugar beet seedlings under hydro-thermal priming using the Pearson method.

	Germination percentage (lab)	Emergence percentage (field)	Germination uniformity (lab)	Emergence Uniformity (field)
Germination percentage (lab)	1			
Emergence percentage (field)	0.649**	1		
Germination uniformity (lab)	0.730**	0.752**	1	
Emergence uniformity (field)	0.408 ns	0.662**	0.420 ns	1

*, ** and ns: statistically significant at $p \le 0.05$, $p \le 0.01$ and not significant respectively.

Electrophoresis (SDS-PAGE) Proteins

Four major bands were recorded and it was also observed that the protein profiles of primed and unprimed seeds for two cultivars were the same for these bands (Fig. 1). As a result, hydro-thermal priming did not have an obvious effect on the seed protein patterns of two sugar beet cultivars.

Discussion

Considering the foregoing results, primed sugar beet seeds can rapidly imbibe and revive the seed metabolism, increasing germination rate and uniformity. The higher uniform and germination percentage of primed seeds were due to the activation of a series of germination processes, including imbibitions and nucleic acid synthesis during seed priming processes (33). Also, the acceleration of germination in primed seeds can be attributed to the increasing rate of cell division in these seeds (34) and stimulation of the metabolic activities involved in the initial seed germination phase (35, 36). On the other hand, temperature seems to play an important role in improving the germination process. The positive effects of temperature increase on germination were mostly due to facilitating water absorption by primed seeds and increasing the activity of enzymes such as α -amylase, protease and lipase. These enzymes play an important role in the growth and development of embryo by hydrolysis of macromolecules (37, 38).

These results demonstrate that optimum hydro-thermal priming can be different for each cultivar of sugar beet, which related to specific differences in the response to priming treatments between cultivars. Some seeds respond to priming treatment after short soaking, whereas in others this effect is not clear. The possible reasons for the different reaction to priming treatment could be due to different growth condition in during seed maturation in the field and the variation in depth of seed dormancy. Therefore, seeds of each cultivar can respond differently to priming treatment. This is in agreement with the results observed by Lanteri et al. (13), who found the differences in the reaction to priming among tomato and pepper seeds. Also, if the period of priming treatment is short, the seeds will not have enough time for the molecular and physiological changes, and if this period is prolonged, it can result in



Figure 1. Banding patterns of proteins based on SDS-PAGE. Lanes 1 and 2 are unprimed seeds of Arya and Shokoofa cultivars, respectively. Lanes 3 and 4 are primed seeds of Arya and Shookofa cultivars.

radicle protrusion, which could cause a decrease in seed quality. The reduced seed quality for a too long period of priming treatment was most probably due to reaching a critical stage in the seeds germination process, which can result in the death of the embryo during subsequent drying. Furthermore, this decrease was due to loosening the cap in the pericarp and leaching out the true seed, which leads to an increase in the ratio of empty seeds. Penalosa and Eira (39) stated that the increased period of priming treatment caused a negative effect on germination rate in tomato seeds through leakage of metabolic substances from seeds.

The beneficial effects of priming on the germination capacity and vigor of the seed was most probably due to stimulation of metabolic activities in the embryo i.e., synthesis of nucleic acids (DNA and mRNA), protein production, repair of cell membrane (7, 14, 35) and induction of biochemical changes such as breaking of dormancy, hydrolysis of inhibitors and enzymes activation (40). Numerous studies have been performed to exhibit the considerable effectiveness of hydro-priming on germination and seedling growth in many crop species, e.g., barley (41), chickpea (42), wheat (43) and sugar beet (12).

As previously mentioned, seed treatment was more beneficial in the field conditions and seed priming increased the percentage of emergence, but the response of the sugar beet cultivars to priming was different. Priming in the Shokoofa cultivar had a more positive effect on the percentage of emergence in two years of experiment. The cultivars, in addition to being genetically different, may also have a different growth condition at the time of seed maturation in the field. These differences can result in changes in the thickness of the seed coat, which is effective in water uptake. Perhaps this positive effect due to retaining the physiological changes induced by the seed treatment which can result in improve emergence of primed seeds. More uniformity and earlier emergence were expressed as the positive effects of seed treatment in synchronizing the seed emergence process. The possible reason for the faster emergence of primed seeds is the increased activity of degrading enzymes, such as α -amylase and the enhanced function of the mitochondria (15). According to Rebetzke et al. (44) and Richard and Lukacs (45), the main factors in improving yields are the early emergence of seedlings in the field by accelerating the closure of the plant's canopy. Also, germination percentage in the laboratory had a positive correlation with emergence percentage in the field (0.649^{**}) . These results showed that the percentage of emergence in the field condition can be predicted by determination of seed germination in the laboratory. The same results were reported by Wang et al. (46) and Rehman et al. (47).

The SDS-PAGE banding patterns of the seed proteins did not change by hydro-thermal priming. Also, there was no significant difference in the banding patterns of proteins between two sugar beet cultivars, probably because of low genetic diversity. It can be suggested to utilize more precise approach such as two- dimensional electrophoresis in order to assess the effect of hydrothermal priming on the seed proteins of sugar beet cultivars. The usefulness of this technique has already been reported by (48,49), who used it to detect the protein variations in 46 accessions of groundnut (*Arachis hypogaea*).

In general, this study shows that hydro-thermal priming can increase germination percentage of sugar beet seeds under laboratory condition and improve the emergence in the field. In addition, hydro-thermal priming improves seedling growth rate (seed vigor) which can be attributed to retaining the physiological changes induced by seed priming. This research and similar studies demonstrate that sugar beet cultivars have a positive reaction to seed priming, but the response degree among them is different which it has to be considered in sugar beet seed industry.

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