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Effects of Physical, Mechanical and Hormonal Treatments of Seed-Tubers on Bud Dormancy and Plant Productivity

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Abstract: The aim of this study was to develop a technique easy to apply in order to induce seed-tuber dormancy breakage. Over a two-year study, more than seven dormancy-breaking treatments were tested through evaluating different temperature effects alone or combined with gibberellins application, cutting in half of seed-tubers, and early haulm killing. Three varieties per year were considered: Spunta and Monalisa (medium and long dormancy) in both years, Europa during the first year and Arinda during the second year (both characterized by a short dormancy period). We found firstly that Europa and Arinda promptly responded to thermal treatments, and secondly to the same thermal treatments in combination with the application of gibberellins. Although not easily applicable, especially when a large volume of seed-tubers has to be handled (seed-tuber producers), the cutting in half of the seed-tubers also had a satisfactory result. Notwithstanding that treatments did not perfectly overlap between the two experiments, results were qualitatively similar. Therefore, these findings allow us to conclude that treatment with post-harvest storage at 20 °C, followed by a treatment with gibberellic acid at 38 days from harvesting, is the most efficient in releasing dormancy, in ensuring a good vegetative growth and productive performance at field-level irrespective of the variety.

Keywords: *Solanum tuberosum* L.; gibberellins; abscisic acid; sprouting induction; offseason production; double cropping

1. Introduction

Potato (*Solanum tuberosum* L.) is one of the most important and globally widespread crops [1] being second only to cereals and being grown on more than 19 million hectares in 2017 [2]. In many countries of the Mediterranean basin, potato cultivation is typically characterized by an offseason growing cycle (winter–spring or summer–autumn growing cycle). The offseason product is intended for export to other European countries allowing farmers for profitable prices [3]. Seed-tubers used for planting of the offseason growing cycles have different origins since they may be imported as commercial seed-potatoes from North European countries or may derive from local seed-(re)-producers [4]. Immediately after harvesting, tubers that are going to be used as seeds are not able to sprout due to the bud dormancy and this is a relevant issue if the new sowing has to be done within 2–3 months of harvesting [5].

Dormancy is the full inability to sprout or grow even if environmental factors are at optimal level. Indeed, bud dormancy is affected by several factors such as genetic and environmental ones, tuber

physiological age, storage conditions, chemical and hormonal treatments, tuber cutting or injuries, and early haulm killing [6]. In an open field growing condition, unusual periods of cold or heat during the tuberization stage lead to a shortening or prolongation of dormancy [7]. The prompt interruption of dormancy occurs when temperatures are very hot just before or soon after harvesting. Depending on the variety, temperatures above 35 °C can cause an immediate dormancy breakage and the onset of a psychopathy (heat sprouting) [8]. During post-harvest storage, dormancy may be highly variable also within a seed-lot of tubers belonging to the same variety and this variability may be linked to the degree of maturation of tubers [9]. Indeed, it has been observed that immature tubers have a longer dormancy period compared to tubers harvested at maturity [10,11]. Caldiz [12] reported that tubers have an intrinsic capacity to sprout that gradually rises with age to a peak, and after a long period of storage, it starts to decrease. At storage level, high temperature is considered the main factor that shortens the duration of dormancy promoting sprouting activity [13]. In particular, Struik [14] assessed that during the period of storage, accumulated thermal units control the physiological age, and at equal values of thermal units, the effect is different depending on whether they were accumulated at the beginning or during the final period of storing. At least four phytohormones are known to regulate bud dormancy induction, maintenance and release [7,15]. Genetic analysis highlighted that metabolism, synthesis and signaling of phytohormones, such as abscisic acid, and gibberellins, are regulated by QTL (quantitative traits loci) underlying genes, some of which coincided with QTL underlying genes involved in dormancy and tuberization [16,17]. Moreover, recently Bisognin et al. [18], reported that at least eight QTLs are involved in the termination of dormancy. Full dormancy release is regulated by both gibberellins and cytokinins [15], dormancy induction is promoted by abscisic acid and ethylene, and abscisic acid is considered to be responsible for dormancy maintenance [19]. Moreover, cytokinins and GAs (gibberellins) reactivate meristematic and sprouting activity shortly before dormancy termination [20,21].

Dormancy termination in seed-tuber by exogenous compounds has several practical purposes such as for export or local use, for handle genetic materials of different end-uses (ware or seed) and for planning the next planting season [22]. In particular, seed-tuber producers have an interest in applying techniques that easily and effectively break dormancy allowing the use of seed-tubers for a new growing cycle just few weeks after harvesting [18,23]. Different protocols have been set up to force the end of dormancy. Application of exogenous GA₃ is commonly used to promote dormancy release, even if it was found that the levels of intrinsic GA₃ do not increase until the beginning of tuber sprouting [24]. Eshel and Teper-Bamnolker [25] stated that dormancy release may be also promoted by applying rindite, bromoethane, and carbon disulphide. Cutting of seed-tubers is also known to release or to shorten the period of dormancy [26] and the combination of cutting with GA increases the effect on overcoming tuber dormancy [24]. Moreover, tubers injured by fungal or insect attacks, or wounded by harvesting operations or pre-storage handling, release dormancy earlier than unwounded or healthy tubers [27]. Dormancy lasts about two months and represents a limiting factor if the objective is to use tubers as seed. In the case of potato grown with a summer–autumn cycle it is often necessary to use seed-tubers produced by a usual potato cycle (spring–summer). The study of post-harvest dormancy mechanisms and the response to treatments for dormancy release, therefore, represents a key-point in improving cultivation techniques and the productivity of the offseason potato with summer–autumn cycle.

As far as we know, to date no resolution protocol has been provided concerning the application of dormancy-breaking treatments and their interaction with different varieties in southern Italy. Thus, the main aim was to develop an easy to apply and effective technique to release seed-tuber dormancy. The specific objectives were the following: (1) to establish the most effective combination of factors (temperature, GA application, or cutting) in releasing the bud dormancy in three different varieties, characterized by different dormancy period duration; and (2) to analyze in-field potato crop performance to find a potential relation between treatment applied in the storage phase and potato crop response at the open-field scale.

2. Materials and Methods

Two different and independent experiments were conducted during 1999 (Experiment 1; Exp-1) and 2000 (Experiment 2; Exp-2) at the experimental farm “Palloni” of the Agricultural Research Agency of Sardinia (Oristano, Italy; 39°55′ N, 8°35′ E, 6 m a.s.l.) in the Campidano Plain, one of the most suitable areas for the cultivation of the offseason potato in Southern Italy. The soil is silty-clay loam of alluvial origin (Vertic Xerofluvents; [28]) with pH of 7.2, organic matter content of 25.8 g kg^{−1}, and N, P, K, and Ca content of 1.6 g kg^{−1}, 68.1, 128, and 3783 mg kg^{−1}, respectively.

The climate is Mediterranean with mild winter season and with drought-prone and hot spring–summer season [29]. Each experiment included two phases, the first phase (at storage chamber level) was aimed to test the seed-tubers response to seven dormancy-breaking treatments, in the second one the same seed-tubers were planted and evaluated at open-field growing level. In the period of investigation, total mean rainfall was 254 mm and 276, for 1999 (beginning of September–mid December) and 2000 (end August–half January), respectively (Figure 1b) and it was in line with the long-term average for the same period (288 mm). In 1999 the average temperature was consistent with respect to the long-term series (14.8 vs. 15.5, respectively) from September to December. In 2000 the seasonal thermal trend slightly deviated from the typical averages of the historical series (1979–2009) for the average temperature (−1.1 °C). These differences were more pronounced during the end of summer and autumn period, in fact from the end of August (the period during which potato planting occurred) to November the average temperature was 2.1 °C lower than the historical average.

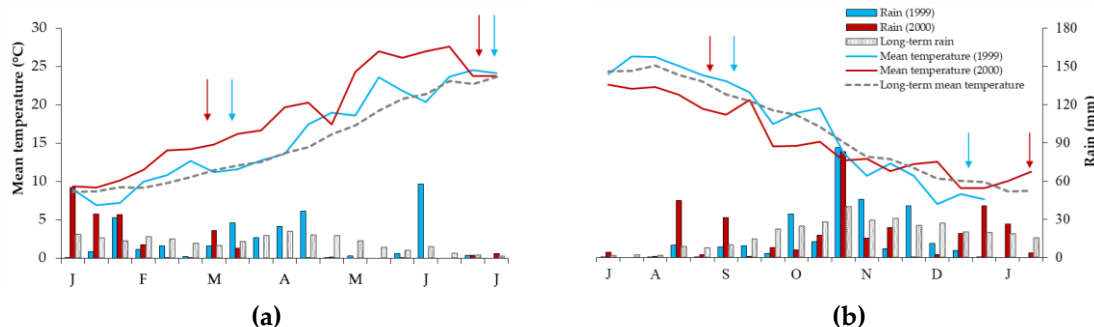


Figure 1. Rainfall (bars) and average temperature (lines) at 10-day interval for Experiment 1 (1999), Experiment 2 (2000) and long-term series (30-year period). Panel (a) refers to the period when seed-tuber mother plants were grown and harvest, panel (b) refers to the period when the same seed-tubers were tested (Experiment 1, and Experiment 2) at open-field level (from the end of August onwards). Arrows represent approximate planting and harvesting dates of seed-tuber mother plants (a) and of experiments at open field level (b) for 1999 (light blue) and 2000 (dark red), respectively.

2.1. Varieties and Dormancy-Breaking Treatments

In each experiment, the choice of varieties was made taking into account their representativeness in terms of duration of the dormancy period. Each year, three varieties with a short-, medium- and a long-dormancy period were tested. In Exp-1 Europa, Monalisa, and Spunta were compared: Europa (Edzina × Alcmaria) produces oval and large tubers and is suitable to be used for offseason potato production; Monalisa (Bierma A1-287 × Colmo) is the most common variety used at local level for offseason production [30,31]; Spunta (Bea × USDA 96–56) is one of the most widespread varieties, early to medium maturity, with tubers long in shape and very large. In Exp-2 Arinda (Vulkano × AR 74–78-1) was considered in place of Europa because of absence of seed-tubers in the multiplication field. According to [32], the names and codes reported between parentheses and after the name of the varieties specify the cross combination of each variety. Arinda, as well as Spunta, is the most widely grown variety for offseason production in the Mediterranean region [3], it has an early maturity period and tubers long to oval in shape and very large. For the production of potatoes, generally,

seed-tubers are the usual means of propagation. If these are ranging from 25 to 60 mm in diameter, they are indicated as conventional seed-tubers [33,34]. In the case of our study, the size was in the range 35–55 mm. On 9 July 1999 (Exp-1) tubers, from a previous on-site trial, were harvested directly without previous haulm killing, placed into dark condition (relative humidity 85.5%) and treated differently to terminate dormancy (Table 1). In T1 treatment seed-tubers were kept at 20 °C for 38 days; in T2 treatment seed-tubers were exposed for 10 days at 10 °C alternating with 4 days at 20 °C; in T3 treatment seed-tubers were exposed 10 days at constant temperature (2 °C) alternating with 4 days at 20 °C; in T4 treatment seed-tubers were exposed 10 days at 38 °C followed by 28 days at 20 °C; in T5 treatment seed-tubers were exposed 38 days at 20 °C and then treated with gibberellic acid (GA₃) at 39 days after harvesting; in T6 treatment seed-tubers were exposed 10 days at 10 °C alternating with 4 days at 20 °C and then treated with GA₃ at 39 days after harvesting; in T7 treatment seed-tubers were exposed 10 days at 2 °C alternating with 4 days at 20 °C and then treated with GA₃ at 39 days after harvesting. For GA₃ treatments, seed-tubers were submerged for 5 min within an aqueous solution of 10 ppm GA₃ concentration (ProGibb 40SG, Sumitomo Chemical Italia srl, Milano, Italy). Upon 38 days after harvesting, seed-tubers were kept at diffuse light and 20 °C for a 22-day period (Table 1). In Exp-1, the total duration of the storage chamber phase was of 60 days.

In the second year (Exp-2), potato seed-tubers of Arinda, Monalisa, and Spunta were obtained from a multiplication field of the research farm. Two different haulm managements were compared. Part of the haulms were removed (R) at 90 days after planting (Table 2), tubers harvesting occurred 10 days later, then tubers were stored at 20 °C in dark conditions until 125 days after planting (30 June) when even tubers of the remaining haulms, left undisturbed (U) in the field, were harvested (Table 2). Seed-tubers originated from different pre-harvest management (R vs. U) were treated differently as follows: R-T1 seed-tubers kept at 20 °C for 58 days, R-T2 seed-tubers treated with GA₃ (aqueous solution 10 ppm) at 79 days after harvesting, U-T3 seed-tubers kept at 20 °C for 58 days, U-T4 seed-tubers treated with GA₃ (aqueous solution 10 ppm) at 54 days after harvesting, U-T5 seed-tubers kept at 20 °C for 48 days followed by a 10-day period at 32 °C, U-T6 seed-tubers kept at 20 °C for 53 days followed by 5 days at 38 °C; U-T7 seed-tubers kept at 20 °C for 48 days; at 49 days after harvesting, tubers were cut in half and kept at 20 °C for a further 10 days. In Exp-2, the total duration of the storage chamber phase was of 58 days.

Table 1. Experiment 1 carried out at seed-tubers level in a storage chamber (1999). GA₃: gibberellic acid application (aqueous solution of 10 ppm); DAH: days after harvesting.

Treatments	Dark Condition						Light Diffuse Condition					
	Temperature (°C)	Duration (days)	Temperature (°C)	Duration (days)	Temperature (°C)	Duration (days)	Temperature (°C)	Duration (days)	Temperature (°C)	Duration (days)	GA ₃	
											(ppm)	(DAH)
T1	20	38	-	-	-	-	-	-	-	-	-	-
T2	10	10	20	4	10	10	20	4	10	10	-	-
T3	2	10	20	4	2	10	20	4	2	10	-	-
T4	38	10	20	28	-	-	-	-	-	-	-	-
T5	20	38	-	-	-	-	-	-	-	-	10	39
T6	10	10	20	4	10	10	20	4	10	10	10	39
T7	2	10	20	4	2	10	20	4	2	10	10	39

Table 2. Experiment 2 (2000). DAP: days after planting; GA₃: gibberellic acid application; DAH: days after harvesting.

Haulms						Tubers							
Cut	Harvest	Post-Harvest Storage				Treatments	Temperature	Duration (days)	GA ₃		Mechanical	Temperature (°C)	Duration (days)
(DAP)	(DAP)	Temperature (°C)	Duration (days)	Light Condition	(ppm)				(DAH)				
Removed (R)	90	100	20	25	Dark	R-T1	20	58	-	-	-	-	-
						R-T2	20	58	10	79	-	-	-
Undisturbed (U)	-	125	-	-	-	U-T3	20	58	-	-	-	-	-
						U-T4	20	58	10	54	-	-	-
						U-T5	20	48	-	-	-	32	10
						U-T6	20	53	-	-	-	38	5
						U-T7	20	48	-	-	Cut in half	20	10

2.2. Absciscic Acid Analysis and Dormancy Release Assessment

During the post-harvest dormancy phase, abscisic acid (ABA) content was determined through the enzyme-linked immunosorbent assay (ELISA) analytical technique [35]. On each tuber, all the buds and the underlying tissue (until a depth of 7 mm) were removed and stored at $-80\text{ }^{\circ}\text{C}$, until analysis was carried out. Freeze-dried samples were finely ground, extracted in aqueous methanol (80% *v/v*) in which BHT (butylated hydroxy-toluene, 0.1% *w/v*) was dissolved, shaken up at $4\text{ }^{\circ}\text{C}$ in the dark and purified through filtration with Whatman paper filters (no. 5). The extracts were assayed in an enzyme-linked immunosorbent assay for ABA using monoclonal antibodies (AFRC MAC252) [36,37]. In Exp-1, three tubers per variety and treatment (replicated three times) were analyzed for ABA content in different time intervals: at seed-tubers harvesting (9 July), 38 days after seed-tubers harvesting (16 August), during the exposure of seed-tubers at diffuse light conditions (23 and 30 August) and before the seed-tubers planting (6 September). In Exp-2, analysis for ABA content was performed in different time intervals and specifically at the harvest of seed-tubers derived from potato plants removed early (5 June), at harvest of seed-tubers derived from undisturbed potato plants (30 June), before exposure to $32\text{ }^{\circ}\text{C}$, and tubers being cut in half (18 August), before exposure to $38\text{ }^{\circ}\text{C}$ and before GA₃ application (24 August) and before seed-tuber planting (29 August). Physiological indicators, such as the sprouting capacity, and the length of the longest sprout were used to establish differences in dormancy release [38]. In Exp-1 on 30 tubers per variety and treatment (three replicates), sprout length measurements were taken and the length of the longest sprout was recorded every week. Tubers were considered to have broken dormancy when average sprout length was $\geq 2\text{ mm}$. In Exp-2, 30 tuber samples per replicate were visually evaluated [39] as regards sprouting activity by using a visual scale (0 full dormancy; 5 full sprouting).

2.3. Experimental Design, Field Management, Observations and Sampling

In both growing seasons, field-level experiments were carried out according to a randomized split plot design replicated three times, with varieties in the main plot and dormancy-breaking treatments in the subplot. Subplot size was 7.5 m^2 with two rows of 20 plants each (5.3 plants m^{-2} planting density). The seed-tubers were planted on 9 September (Exp-1) and on 29 August (Exp-2). Before planting 60 kg N ha^{-1} , $150\text{ kg P}_2\text{O}_5\text{ ha}^{-1}$, $150\text{ kg K}_2\text{O ha}^{-1}$ (NPK fertilizer; 8-24-24, Adriatica S.p.A., Loreo, Italy) and $300\text{ kg CaO ha}^{-1}$ (30%, Cooperativa agricola Spazio-soc. coop., Signoressa di Trevignano, Italy) were applied. In mid-October, earthing up was performed and an additional 20 kg N ha^{-1} (ammonium nitrate; 26%, Yara Italia S.p.A., Milan, Italy) was supplied. Weed control occurred during the crop critical stages by applying Metribuzin (Feinzin 70 DF, Adama Italia srl, Bergamo, Italy). In Exp-1, emergence (number of plants per plot) was recorded from 6 October to 10 November, and number of stems per plant was counted from 12 October to 13 December. In Exp-2, plant emergence was assessed by counting the number of plants that emerged (%) per plot on 15 September. Harvest occurred on average 119 days after planting (13 December and 17 January in Exp-1 and Exp-2, respectively) when more than 50% of haulms have senesced. At harvest, 20 plants per treatment were collected. For determining marketable yield, only tubers with a diameter between 35 and 70 mm were counted and weighted, whereas tubers with anomalies were considered as unmarketable.

2.4. Statistical Analysis

Analysis of variance (ANOVA) with a split-plot design was used to assess the significant impacts of varieties and breaking-dormancy treatments and their interactions on tuber and crop variables. Variety and breaking-dormancy treatment were considered as fixed effect; block and interaction with block as random effect. Therefore, the PROC MIXED model was used [40] with Tukey's honest significant difference (HSD) test for the comparison of mean values at $p < 0.05$, unless stated otherwise. When two treatments were considered (e.g., Removed vs. Undisturbed in Exp-2) significance was tested by the Student T-test ($p < 0.05$ level). Normalized data were then subjected to principal component analysis

(PCA) to study which parameters contributed most to the total data variation and it was performed with R software [41].

3. Results

3.1. Experiment 1 (Exp-1)

At the beginning of the trial, before treatments application, ABA content was on average equal to 15 ng g⁻¹ fresh weight with no significant differences among varieties. After the application of thermal and hormonal treatments, ABA content shows a decreasing trend. At the last analysis date, the ABA content was, however, consistent among treatments and on average equal to 3 ng g⁻¹ fresh weight. Specifically, after thermal treatments applied on 16 August, a relevant decrease in ABA content (3.9 ng g⁻¹ of fresh weight in T4 treatment and 5.8 ng g⁻¹ of fresh weight in T1 treatment) was observed in the highest thermal levels (Table 3). The treatment that alternated 2 °C with 20 °C (T3) did not significantly affect ABA content even when it was combined with GA₃ application (T7). Monalisa showed an ABA content statistically higher compared to the other two varieties.

The results (Table 4) highlighted a progressively increasing sprout activity starting from the first monitoring date. T4 treatment, in particular, was able to stimulate the highest tuber sprouting activity by showing during the entire duration of monitoring the highest sprouts number compared with the other treatments (on average 3.5 sprouts longer than 2 mm per data of observation). Only at the 3rd date of monitoring, tubers of T5–T7 treatments showed statistically greater number of sprouts longer than 2 mm when compared with the treatments characterized by the same temperature level, but without applying exogenous GA₃ (T1–T3). Monalisa variety was characterized by a lower number of sprouts per tuber, whereas Europa, at the last monitoring date, showed a significantly higher number of sprouts if compared with Spunta.

The monitoring showed a stable pattern in the four dates as T4 was the treatment that significantly affected the length of the longest sprout (on average higher than 10 mm). T1 and T5 ranked second as the treatments that promote a longest sprouts length, with no significant difference from T4 at the last monitoring date. At the first date of monitoring Europa tubers subjected to T4 treatment showed sprouts of 24 mm. By contrast, Monalisa, being characterized by a slower vegetative activity, showed sprout length rarely higher than 2.5 mm (Table 5).

T4–T7 treatments had the highest percent of plant emergence in the second monitoring date (12 October). Indeed, from 12 October onwards a statistically lower plant emergence percent was shown only for T4 treatment that at the last observation date showed about 60% of emerged plants (Table 6). Europa variety showed an increasing percent of plant emergence (Table 6) from the second to the last date of observation, by contrast Monalisa showed significant lower plant emergence values.

T4, T5, T6 and T7 originated plants with the highest number of stems. At the harvesting time, only T4 treatment showed an average of 2 stems per plant (Table 7). The number of stems per plant was also always higher in Europa, whereas Monalisa had the lowest stem number, in all observation dates.

At harvesting time, T4 treatment negatively affected tuberization phase since in fact the plant produced only 2.89 commercial tubers per plant. By contrast, the remaining treatments did not significantly differ with an average tuber production equal to 5 tubers per plant. A similar pattern was also observed for tuber weight per plant. Europa showed the highest number of commercial tubers per plants (Table 8).

3.2. Exp-2

Results showed a high ABA content at harvest (Table 9) in tubers from plants whose aerial part was removed 10 days before harvesting (see 5 June). In these tubers the ABA content progressively decreased, as highlight by ABA content of samples taken on 30 June, simultaneously to the harvest time of plants whose aerial part was left undisturbed (Table 9).

Table 3. Average (\pm standard error) abscisic acid (ABA) content (ng g⁻¹ fresh weight) of three varieties (Europa, Monalisa and Spunta) subjected to seven dormancy-breaking treatments applied during pre-planting storage condition (Experiment 1).

Treatments	9-Jul			16-Aug			23-Aug			30-Aug			06-Sep		
	Eur	Mon	Spu	Eur	Mon	Spu	Eur	Mon	Spu	Eur	Mon	Spu	Eur	Mon	Spu
T1		–		3.8 \pm 0.1	9.3 \pm 0.5	5.0 \pm 0.4	2.5 \pm 0.1	3.3 \pm 0.2	2.7 \pm 0.2	2.0 \pm 0.04	3.0 \pm 0.1	2.8 \pm 0.4	2.5 \pm 0.1	2.9 \pm 0.1	2.5 \pm 0.1
T2		–		5.7 \pm 0.2	10.3 \pm 1.0	6.0 \pm 0.2	4.0 \pm 0.1	4.0 \pm 0.1	3.3 \pm 0.1	3.0 \pm 0.1	3.5 \pm 0.02	3.0 \pm 0.03	2.5 \pm 0.1	3.4 \pm 0.2	2.6 \pm 0.02
T3		–		6.5 \pm 0.3	8.3 \pm 0.3	5.3 \pm 0.2	5.5 \pm 0.2	4.8 \pm 0.1	6.0 \pm 0.4	4.5 \pm 0.1	4.0 \pm 0.1	4.5 \pm 0.1	3.2 \pm 0.2	3.6 \pm 0.2	3.3 \pm 0.01
T4		–		3.7 \pm 0.3	4.7 \pm 0.4	3.5 \pm 0.1	–	–	–	–	–	–	–	–	–
T5		–		–	–	–	2.8 \pm 0.6	3.8 \pm 0.3	3.0 \pm 0.1	2.5 \pm 0.04	3.5 \pm 0.1	2.7 \pm 0.1	2.4 \pm 0.2	2.8 \pm 0.1	2.5 \pm 0.1
T6		–		–	–	–	3.8 \pm 0.2	4.8 \pm 0.1	3.7 \pm 0.1	3.0 \pm 0.1	3.3 \pm 0.1	2.8 \pm 0.1	2.6 \pm 0.1	3.0 \pm 0.1	2.4 \pm 0.1
T7		–		–	–	–	5.3 \pm 0.5	5.3 \pm 0.2	5.8 \pm 0.2	4.3 \pm 0.1	3.8 \pm 0.1	4.7 \pm 0.3	3.7 \pm 0.1	3.3 \pm 0.2	3.4 \pm 0.04
Significance		$p > F$			$p > F$			$p > F$			$p > F$			$p > F$	
Variety (V)		0.385			0.028			0.932			0.213			0.098	
Treatment (T)		–			0.825			<0.001			0.041			0.004	
V \times T		–			0.776			0.888			0.249			0.071	

– “En Dash” means that on 9 July (T1–T7) and on 16 August (T5–T7) breaking-dormancy treatments were not yet imposed, whereas between 23 August and 6 September, it means that in T4 treatment dormancy was already broken and no further ABA analysis was performed. Eur, Mon, Spu: Europa, Monalisa, Spunta varieties, respectively. T1: 20 °C; T2: 10/20 °C; T3: 2/20 °C; T4: 38/20 °C; T5: 20 °C + GA₃; T6: 10/20 °C + GA₃; T7: 2/20 °C + GA₃.

Table 4. Average (\pm standard error) number of sprouts (no.) per tuber with a minimum length of 2 mm of three varieties (Europa, Monalisa, Spunta) subjected to seven dormancy-breaking treatments applied during pre-planting storage condition (Experiment 1).

Treatments	16-Aug			23-Aug			30-Aug			06-Sep		
	Eur	Mon	Spu	Eur	Mon	Spu	Eur	Mon	Spu	Eur	Mon	Spu
T1	0.7 \pm 0.01	0.3 \pm 0.02	0.6 \pm 0.01	0.8 \pm 0.06	0.3 \pm 0.01	0.6 \pm 0.01	1.7 \pm 0.31	0.7 \pm 0.02	0.9 \pm 0.05	2.7 \pm 0.33	1.1 \pm 0.06	2.1 \pm 0.08
T2	0.2 \pm 0.01	0.2 \pm 0.01	0.2 \pm 0.01	0.3 \pm 0.01	0.3 \pm 0.01	0.3 \pm 0.01	0.7 \pm 0.01	0.3 \pm 0.01	0.4 \pm 0.01	2.5 \pm 0.50	0.7 \pm 0.02	1.6 \pm 0.08
T3	0.0 \pm 0.01	0.0 \pm 0.01	0.0 \pm 0.01	0.0 \pm 0.02	0.0 \pm 0.01	0.0 \pm 0.02	0.7 \pm 0.01	0.1 \pm 0.01	0.7 \pm 0.03	2.1 \pm 0.12	0.6 \pm 0.01	1.3 \pm 0.06
T4	4.9 \pm 0.22	0.7 \pm 0.01	4.8 \pm 0.24	5.3 \pm 0.10	0.7 \pm 0.24	4.7 \pm 0.22	5.6 \pm 2.94	1.3 \pm 0.81	4.3 \pm 1.30	5.5 \pm 0.76	1.3 \pm 0.19	3.8 \pm 0.17
T5	–	–	–	0.9 \pm 0.01	0.4 \pm 0.01	0.7 \pm 0.01	3.1 \pm 0.40	0.9 \pm 0.01	2.0 \pm 0.53	3.5 \pm 0.48	1.2 \pm 0.06	3.2 \pm 0.20
T6	–	–	–	0.2 \pm 0.01	0.2 \pm 0.01	0.2 \pm 0.01	2.3 \pm 0.25	0.4 \pm 0.01	1.7 \pm 0.90	3.2 \pm 0.60	1.6 \pm 0.08	2.7 \pm 0.16
T7	–	–	–	0.0 \pm 0.01	0.0 \pm 0.01	0.1 \pm 0.01	2.2 \pm 0.48	0.6 \pm 0.01	1.6 \pm 0.42	3.3 \pm 0.36	1.4 \pm 0.07	2.3 \pm 0.86
Significance		$p > F$			$p > F$			$p > F$			$p > F$	
Variety (V)		<0.001			<0.001			<0.001			<0.001	
Treatment (T)		<0.001			<0.001			<0.001			<0.001	
V \times T		<0.001			<0.001			<0.001			<0.001	

– “En Dash” means that on 16 August T5–T7 treatments were not yet imposed. Eur, Mon, Spu: Europa, Monalisa, Spunta varieties, respectively. T1: 20 °C; T2: 10/20 °C; T3: 2/20 °C; T4: 38/20 °C; T5: 20 °C + GA₃; T6: 10/20 °C + GA₃; T7: 2/20 °C + GA₃.

Table 5. Average (\pm standard error) length of the longest sprout (mm) of three varieties (Europa, Monalisa, Spunta) subjected to seven dormancy-breaking treatments applied during pre-planting storage condition (Experiment 1).

Treatments	16-Aug			23-Aug			30-Aug			06-Sep		
	Eur	Mon	Spu	Eur	Mon	Spu	Eur	Mon	Spu	Eur	Mon	Spu
T1	16.4 \pm 2.13	0.9 \pm 0.01	10.6 \pm 3.05	15.1 \pm 1.07	1.7 \pm 0.01	10.8 \pm 2.21	15.8 \pm 2.78	2.6 \pm 0.55	9.7 \pm 0.82	17.9 \pm 2.22	4.3 \pm 0.36	11.4 \pm 2.01
T2	1.1 \pm 0.09	0.8 \pm 0.01	1.0 \pm 0.01	1.4 \pm 0.02	1.1 \pm 0.01	1.2 \pm 0.01	2.1 \pm 0.20	1.4 \pm 0.46	1.4 \pm 0.11	5.1 \pm 0.71	2.6 \pm 0.38	4.3 \pm 0.44
T3	0.2 \pm 0.01	0.1 \pm 0.01	0.0 \pm 0.01	0.3 \pm 0.01	0.2 \pm 0.01	0.0 \pm 0.01	1.7 \pm 0.72	0.4 \pm 0.01	1.6 \pm 0.51	4.8 \pm 0.98	1.7 \pm 0.05	3.3 \pm 0.03
T4	23.7 \pm 7.88	2.0 \pm 0.44	12.6 \pm 2.53	23.7 \pm 4.11	2.8 \pm 0.71	13.7 \pm 2.76	20.3 \pm 2.33	4.0 \pm 0.26	13.0 \pm 1.25	19.9 \pm 2.34	4.2 \pm 0.74	11.4 \pm 1.91
T5	–	–	–	7.2 \pm 1.20	3.0 \pm 0.34	4.5 \pm 1.71	12.9 \pm 1.98	5.0 \pm 0.79	11.4 \pm 2.18	16.0 \pm 3.12	5.7 \pm 0.88	15.1 \pm 1.79
T6	–	–	–	0.8 \pm 0.01	0.6 \pm 0.01	0.8 \pm 0.01	4.7 \pm 0.44	1.8 \pm 0.11	6.3 \pm 1.00	8.6 \pm 0.45	4.5 \pm 0.09	13.5 \pm 2.01
T7	–	–	–	0.0 \pm 0.01	0.5 \pm 0.01	0.4 \pm 0.01	3.8 \pm 0.51	2.6 \pm 0.09	5.0 \pm 0.75	7.6 \pm 0.61	5.6 \pm 0.33	8.8 \pm 1.08
Significance		$p > F$			$p > F$			$p > F$			$p > F$	
Variety (V)		<0.001			<0.001			<0.001			<0.001	
Treatment (T)		<0.001			<0.001			<0.001			<0.001	
V \times T		<0.001			<0.001			<0.001			<0.001	

– “En Dash” means that on 16 August T5–T7 treatments were not yet imposed. Eur, Mon, Spu: Europa, Monalisa, Spunta varieties, respectively. T1: 20 °C; T2: 10/20 °C; T3: 2/20 °C; T4: 38/20 °C; T5: 20 °C + GA₃; T6: 10/20 °C + GA₃; T7: 2/20 °C + GA₃.

Table 6. Average (\pm standard error) percentage of plant emergence (%) in open-field condition for three varieties (Europa, Monalisa and Spunta) subjected to seven pre-planting dormancy-breaking treatments and across different monitoring dates (Experiment 1).

Treatments	06-Oct			12-Oct			22-Oct			29-Oct			10-Nov		
	Eur	Mon	Spu	Eur	Mon	Spu	Eur	Mon	Spu	Eur	Mon	Spu	Eur	Mon	Spu
T1	5 \pm 0.72	0 \pm 0.01	16 \pm 1.41	40 \pm 4.35	0 \pm 0.01	47 \pm 6.15	93 \pm 14.6	47 \pm 2.98	73 \pm 12.0	100 \pm 22.3	93 \pm 21.0	73 \pm 12.3	100 \pm 11.6	93 \pm 11.1	80 \pm 8.74
T2	0 \pm 0.01	0 \pm 0.01	5 \pm 0.63	13 \pm 1.19	0 \pm 0.01	13 \pm 1.11	93 \pm 11.3	20 \pm 2.07	87 \pm 12.7	93 \pm 8.64	87 \pm 8.84	87 \pm 9.6	93 \pm 8.12	100 \pm 7.01	87 \pm 6.11
T3	0 \pm 0.01	0 \pm 0.01	4 \pm 0.11	13 \pm 1.02	0 \pm 0.01	27 \pm 1.67	73 \pm 9.12	33 \pm 1.91	80 \pm 11.4	87 \pm 17.1	93 \pm 24.3	93 \pm 13.7	87 \pm 17.7	100 \pm 14.6	100 \pm 10.2
T4	71 \pm 12.1	2 \pm 0.08	58 \pm 9.33	67 \pm 11.4	13 \pm 2.01	67 \pm 8.36	73 \pm 4.57	20 \pm 1.46	80 \pm 9.36	73 \pm 3.79	27 \pm 3.17	80 \pm 11.2	73 \pm 3.11	33 \pm 1.50	80 \pm 10.1
T5	80 \pm 14.6	29 \pm 1.44	78 \pm 11.0	80 \pm 13.3	73 \pm 11.9	80 \pm 10.9	87 \pm 10.2	80 \pm 15.2	87 \pm 8.44	93 \pm 10.4	87 \pm 12.1	93 \pm 18.4	93 \pm 7.12	100 \pm 8.91	93 \pm 10.3
T6	36 \pm 1.75	18 \pm 3.52	80 \pm 11.4	87 \pm 10.1	53 \pm 8.01	87 \pm 16.7	93 \pm 17.4	93 \pm 15.5	73 \pm 18.2	93 \pm 9.04	100 \pm 19.1	87 \pm 14.5	100 \pm 25.0	100 \pm 15.2	87 \pm 9.61
T7	33 \pm 1.83	9 \pm 0.51	42 \pm 6.62	100 \pm 21.4	47 \pm 4.17	73 \pm 8.84	100 \pm 16.0	93 \pm 16.0	93 \pm 16.9	100 \pm 11.6	93 \pm 12.8	93 \pm 8.74	100 \pm 8.0	93 \pm 9.45	100 \pm 7.65
Significance		$p > F$			$p > F$			$p > F$			$p > F$			$p > F$	
Variety (V)		<0.001			0.002			0.008			0.088			1.527	
Treatment (T)		<0.001			0.035			0.019			0.033			0.047	
V \times T		<0.001			1.645			0.072			0.577			0.645	

Eur, Mon, Spu: Europa, Monalisa, Spunta varieties, respectively. T1: 20 °C; T2: 10/20 °C; T3: 2/20 °C; T4: 38/20 °C; T5: 20 °C + GA₃; T6: 10/20 °C + GA₃; T7: 2/20 °C + GA₃.

Table 7. Average (\pm standard error) number of stems per plant (no.) of three varieties (Europa, Monalisa, and Spunta) subjected to seven pre-planting dormancy-breaking treatments and accross different monitoring dates (Experiment 1).

Treatments	12-Oct			25-Oct			29-Oct			10-Nov			13-Dec		
	Eur	Mon	Spu	Eur	Mon	Spu	Eur	Mon	Spu	Eur	Mon	Spu	Eur	Mon	Spu
T1	0.0 \pm 0.01	0.0 \pm 0.01	1.0 \pm 0.01	0.9 \pm 0.01	0.6 \pm 0.01	1.2 \pm 0.09	1.3 \pm 0.02	0.9 \pm 0.01	1.2 \pm 0.07	1.7 \pm 0.14	1.6 \pm 0.09	1.3 \pm 0.05	1.5 \pm 0.21	1.3 \pm 0.10	1.3 \pm 0.07
T2	0.0 \pm 0.01	0.0 \pm 0.01	0.6 \pm 0.01	0.9 \pm 0.01	0.5 \pm 0.01	1.2 \pm 0.07	1.2 \pm 0.01	1.1 \pm 0.02	1.3 \pm 0.08	1.6 \pm 0.11	1.6 \pm 0.13	1.4 \pm 0.06	1.4 \pm 0.09	1.2 \pm 0.08	1.3 \pm 0.09
T3	0.0 \pm 0.01	0.0 \pm 0.01	0.3 \pm 0.01	0.6 \pm 0.01	0.5 \pm 0.01	1.0 \pm 0.09	1.1 \pm 0.50	1.0 \pm 0.02	1.1 \pm 0.13	1.5 \pm 0.13	1.3 \pm 0.05	1.3 \pm 0.10	1.3 \pm 0.07	1.2 \pm 0.08	1.2 \pm 0.10
T4	2.3 \pm 0.11	0.1 \pm 0.01	1.4 \pm 0.11	2.8 \pm 0.12	0.4 \pm 0.01	1.5 \pm 0.06	2.9 \pm 0.14	0.6 \pm 0.01	1.5 \pm 0.05	3.0 \pm 0.15	0.6 \pm 0.01	1.5 \pm 0.11	2.4 \pm 0.11	1.7 \pm 0.11	2.0 \pm 0.13
T5	2.0 \pm 0.10	0.8 \pm 0.05	1.3 \pm 0.09	2.3 \pm 0.10	1.2 \pm 0.02	1.4 \pm 0.14	2.2 \pm 0.11	1.5 \pm 0.08	1.4 \pm 0.11	2.3 \pm 0.10	7.5 \pm 0.22	1.4 \pm 0.09	1.8 \pm 0.12	1.4 \pm 0.07	1.3 \pm 0.11
T6	1.6 \pm 0.05	0.7 \pm 0.07	1.6 \pm 0.08	1.9 \pm 0.11	1.7 \pm 0.14	1.7 \pm 0.12	2.0 \pm 0.09	1.8 \pm 0.08	1.7 \pm 0.09	2.1 \pm 0.08	1.8 \pm 0.09	1.7 \pm 0.11	1.9 \pm 0.21	1.4 \pm 0.07	1.6 \pm 0.09
T7	1.2 \pm 0.05	0.7 \pm 0.08	1.1 \pm 0.04	1.6 \pm 0.08	1.4 \pm 0.11	1.5 \pm 0.08	1.9 \pm 0.15	1.5 \pm 0.08	1.5 \pm 0.12	2.0 \pm 0.07	1.7 \pm 0.12	1.5 \pm 0.12	1.8 \pm 0.14	1.1 \pm 0.01	1.3 \pm 0.08
Significance	$p > F$			$p > F$			$p > F$			$p > F$			$p > F$		
Variety (V)	0.003			0.017			0.020			0.009			0.009		
Treatment (T)	0.043			0.048			0.022			0.038			0.047		
V \times T	0.025			0.015			1.438			0.047			0.065		

Eur, Mon, Spu: Europa, Monalisa, Spunta varieties, respectively. T1: 20 °C; T2: 10/20 °C; T3: 2/20 °C; T4: 38/20 °C; T5: 20 °C + GA₃; T6: 10/20 °C + GA₃; T7: 2/20 °C + GA₃.

Table 8. Average (\pm standard error) marketable and unmarketable plant production (expressed as number and grams of tuber per plant) of three varieties (Europa, Monalisa, and Spunta) subjected to seven dormancy-breaking treatments applied during storage pre-planting condition (Experiment 1).

Treatments	Marketable Yield						Unmarketable Yield					
	Tuber Number (no. plant ⁻¹)			Tuber Weight (g plant ⁻¹)			Tuber Number (no. plant ⁻¹)			Tuber Weight (g plant ⁻¹)		
	Eur	Mon	Spu	Eur	Mon	Spu	Eur	Mon	Spu	Eur	Mon	Spu
T1	5.1 \pm 0.4	5.1 \pm 0.1	4.2 \pm 0.1	268.9 \pm 21	212.6 \pm 21	352.0 \pm 16	0.6 \pm 0.01	0.1 \pm 0.01	0.5 \pm 0.01	27.8 \pm 1.1	5.2 \pm 0.1	99.6 \pm 7.4
T2	5.7 \pm 0.5	4.1 \pm 0.2	4.6 \pm 0.1	259.3 \pm 15	204.2 \pm 18	301.8 \pm 15	0.4 \pm 0.01	0.1 \pm 0.01	0.7 \pm 0.01	23.0 \pm 1.3	5.4 \pm 0.3	64.0 \pm 5.1
T3	5.2 \pm 0.4	4.9 \pm 0.4	4.3 \pm 0.4	242.9 \pm 20	224.6 \pm 15	307.6 \pm 18	0.6 \pm 0.01	0.1 \pm 0.01	0.3 \pm 0.01	45.5 \pm 1.4	2.0 \pm 0.1	38.8 \pm 3.6
T4	4.6 \pm 0.3	1.2 \pm 0.0	3.0 \pm 0.1	227.2 \pm 12	51.4 \pm 5.1	278.1 \pm 20	0.8 \pm 0.01	0.0 \pm 0.01	0.2 \pm 0.01	39.5 \pm 1.4	3.6 \pm 0.1	28.2 \pm 2.0
T5	5.7 \pm 0.1	4.1 \pm 0.4	4.0 \pm 0.2	275.8 \pm 14	272.9 \pm 18	347.9 \pm 25	0.4 \pm 0.01	0.1 \pm 0.01	0.3 \pm 0.01	35.6 \pm 1.6	8.4 \pm 0.5	47.3 \pm 5.7
T6	6.2 \pm 0.2	5.0 \pm 0.1	4.0 \pm 0.1	354.5 \pm 24	316.0 \pm 23	395.5 \pm 21	0.7 \pm 0.01	0.2 \pm 0.01	0.3 \pm 0.01	59.4 \pm 2.0	1.5 \pm 0.1	32.6 \pm 3.8
T7	5.0 \pm 0.1	4.5 \pm 0.1	4.3 \pm 0.1	247.1 \pm 22	470.8 \pm 28	314.3 \pm 24	0.6 \pm 0.01	0.1 \pm 0.01	0.4 \pm 0.01	49.2 \pm 1.4	0.0 \pm 0.1	48.8 \pm 4.4
Significance	$p > F$			$p > F$			$p > F$			$p > F$		
Variety (V)	0.028			0.018			0.003			<0.001		
Treatment (T)	0.037			0.031			0.317			0.076		
V \times T	<0.001			<0.001			0.190			0.087		

Eur, Mon, Spu: Europa, Monalisa, Spunta varieties, respectively. T1: 20 °C; T2: 10/20 °C; T3: 2/20 °C; T4: 38/20 °C; T5: 20 °C + GA₃; T6: 10/20 °C + GA₃; T7: 2/20 °C + GA.

Table 9. Average (\pm standard error) ABA content (ng g^{-1} fresh weight) in tubers of plants early “Removed”, and in tubers of plants left “Undisturbed” on field until the date of harvesting (Experiment 2).

Treatments	5-Jun			30-Jun			18-Aug		
	Ari	Mon	Spu	Ari	Mon	Spu	Ari	Mon	Spu
Removed	8.8 \pm 1.4	9.8 \pm 2.2	9.3 \pm 2.1	4.3 \pm 0.9	5.5 \pm 0.5	4.7 \pm 0.4	2.8 \pm 0.1	3.0 \pm 0.1	3.3 \pm 0.1
Undisturbed	–	–	–	5.3 \pm 1.0	5.3 \pm 0.4	5.3 \pm 0.5	2.3 \pm 0.1	2.5 \pm 0.1	2.3 \pm 0.1
Significance		$p > F$			$p > F$			$p > F$	
Variety (V)		0.212			0.079			0.641	
Treatment (T)		–			0.726			0.047	
V \times T		–			0.064			0.907	

– “En Dash” means that on 5 June only seed-tubers originated from early removed haulms were analyzed for ABA content. Ari, Mon, Spu: Arinda, Monalisa, Spunta varieties, respectively.

About a month and a half later (analysis of 18 August), ABA content of tubers stored in dark condition at 20 °C showed a significant decline with respect to the tubers produced by the plants subjected to the cutting of the aerial part that maintained statistically higher abscisic acid content (Table 10). About 6 days later, the concentration of abscisic acid in the tubers did not undergo significant changes, not showing statistically significant differences with the tubers already subjected to 6 days at a constant temperature of 32 °C (U-T5). At the sampling of 29 August, before the use as seed, no statistically significant difference was highlighted among the tubers of the three varieties; the tubers of plants left undisturbed till harvest, stored at 20 °C (U-T3) or subjected to sectioning (U-T7) had the lowest ABA content (Table 10).

Table 10. Average (\pm standard error) ABA content (ng g^{-1} fresh weight) in seed-tubers subjected to seven dormancy-breaking treatments. Abscisic acid analysis was done before and after the application of gibberellic acid (Experiment 2).

Treatments	24-Aug			29-Aug		
	Ari	Mon	Spu	Ari	Mon	Spu
R-T1	2.3 \pm 0.1	3.0 \pm 0.2	3.5 \pm 0.2	2.3 \pm 0.1	2.0 \pm 0.1	2.3 \pm 0.2
R-T2	–	–	–	2.5 \pm 0.1	2.0 \pm 0.1	1.8 \pm 0.1
U-T3	2.3 \pm 0.1	2.5 \pm 0.1	2.3 \pm 0.1	1.5 \pm 0.1	2.0 \pm 0.1	1.3 \pm 0.1
U-T4	–	–	–	1.8 \pm 0.1	2.0 \pm 0.1	1.5 \pm 0.1
U-T5	1.8 \pm 0.1	2.3 \pm 0.1	2.5 \pm 0.1	2.0 \pm 0.1	2.3 \pm 0.1	2.5 \pm 0.3
U-T6	–	–	–	1.3 \pm 0.1	2.3 \pm 0.1	2.0 \pm 0.1
U-T7	2.0 \pm 0.1	2.3 \pm 0.1	2.0 \pm 0.1	1.0 \pm 0.1	2.0 \pm 0.1	2.0 \pm 0.1
Significance		$p > F$			$p > F$	
Variety (V)		0.517			0.385	
Treatment (T)		0.033			0.029	
V \times T		0.835			0.273	

– “En Dash” means that on 24 August R-T2, U-T4, and U-T6 treatments were not yet imposed. Ari, Mon, Spu: Arinda, Monalisa, Spunta varieties, respectively. R-T1: removed + 20 °C; R-T2: removed + 20 °C + GA₃; U-T3: undisturbed + 20 °C; U-T4: undisturbed + 20 °C + GA₃; U-T5: undisturbed + 32 °C; U-T6: undisturbed + 38 °C; U-T7: undisturbed + cut in half.

Before being used as seed-tubers, U-T7, U-T3 and U-T4 showed a more evident sprouting activity (Table 11). The effect of high temperature (38 °C) on the dormancy of seed-tubers was not statistically significant, likely due to the reduced exposure time (5 days) before their use. The tubers Arinda, Spunta and Monalisa showed a gradually decreasing sprouting activity. At the monitoring date of 15 September, Arinda showed the highest percentage of emerged plants (about 40%). Statistical difference was also observed over treatments, with plots sown with T4 and T7 seed-tubers which presented the highest percentage of emerged plants (Table 11).

Arinda variety produced the highest number of tubers per plant compared to Monalisa and Spunta (Table 12). The tubers of T4 had plants of significantly greater productivity, both in terms of the number of tubers per plant and of the total weight of commercial product per plant.

Unmarketable production did not show significant differences among varieties, whereas the T4 treatment had the highest number of tubers and the highest total weight of non-marketable production per plant (Table 12).

Table 11. In Experiment 2, average (\pm standard error) sprouting activity ranked with the use of a visual scale from 0 (full dormancy) to 5 (full sprouting), and average (\pm standard error) plant emergence (%) at the monitoring date of September 15 (24 days after planting).

Treatments	Sprouting Activity (0–5)			Plant Emergence (%)		
	Ari	Mon	Spu	Ari	Mon	Spu
R-T1	3.0 \pm 0.3	1.0 \pm 0.1	2.0 \pm 0.1	31.3 \pm 4.3	0.0 \pm 0.01	0.0 \pm 0.01
R-T2	2.0 \pm 0.1	1.0 \pm 0.1	2.0 \pm 0.1	35.3 \pm 5.1	0.0 \pm 0.01	18.0 \pm 2.7
U-T3	3.0 \pm 0.2	1.0 \pm 0.1	4.0 \pm 0.2	40.0 \pm 6.0	0.0 \pm 0.01	20.0 \pm 4.4
U-T4	5.0 \pm 0.3	2.0 \pm 0.1	5.0 \pm 0.3	71.3 \pm 10.2	0.0 \pm 0.01	48.7 \pm 7.8
U-T5	2.0 \pm 0.1	1.0 \pm 0.1	1.0 \pm 0.1	20.0 \pm 4.5	0.0 \pm 0.01	4.7 \pm 3.5
U-T6	4.0 \pm 0.2	1.0 \pm 0.1	2.0 \pm 0.1	38.0 \pm 5.8	0.0 \pm 0.01	2.0 \pm 0.1
U-T7	5.0 \pm 0.3	2.0 \pm 0.1	2.0 \pm 0.1	58.0 \pm 6.6	0.0 \pm 0.01	22.0 \pm 5.2
Significance		$p > F$			$p > F$	
Variety (V)		<0.001			<0.001	
Treatment (T)		<0.001			0.0013	
V \times T		0.0831			0.2191	

Ari, Mon, Spu: Arinda, Monalisa, Spunta varieties, respectively. R-T1: removed + 20 °C; R-T2: removed + 20 °C + GA₃; U-T3: undisturbed + 20 °C; U-T4: undisturbed + 20 °C + GA₃; U-T5: undisturbed + 32 °C; U-T6: undisturbed + 38 °C; U-T7: undisturbed + cut in half.

Principal Component Analysis (PCA)

The first two PCs (Figure 2) explained 61.7% of the total variability of the data, which is sufficient to interpret the complex interactions existing between treatments (varieties and breaking-dormancy treatments) and their effects on the tuber and on the crop. Spunta, Arinda and U-T4 and U-T7 treatments are almost arranged along PC1, which accounts for 43.7% of the entire variability, and according to an increasing gradient in terms of dormancy duration from the short-dormant variety (Arinda) to the long-dormant one (Monalisa). The production variables, sprouting activity and plant emergence are grouped in the right part of the biplot and are positively correlated with U-T4, U-T7, Arinda, and Spunta. Therefore, sprouting activity, plant emergence, and marketable production, are favored by these treatments. This is justified by the length of the vectors: the greatest treatment effect corresponds to the longest vector. Moreover, treatments positioned in the right part of the biplot allow the ABA content to be reduced (left part) which is greater in the long-dormant variety Monalisa and in R-T2. Indeed, ABA content vector opposite to U-T4, U-T7, and Spunta highlight a negative correlation. The beneficial effects of U-T4, U-T7, Arinda and Spunta are counterbalanced by a great number of unmarketable tubers.

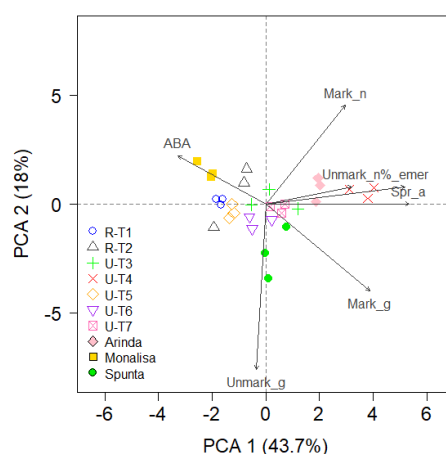


Figure 2. PCA biplot showing patterns and interaction between variables and treatments for Experiment 2. ABA, abscisic acid content; Sprout_a, sprouting activity; %_emer, percentage of plant emergence; Mark_g, grams of tuber per plant; Mark_n, number of tuber per plant; Unmark_g and Unmark_n, grams and number of unmarketable tuber per plant, respectively. R-T1: removed + 20 °C; R-T2: removed + 20 °C + GA₃; U-T3: undisturbed + 20 °C; U-T4: undisturbed + 20 °C + GA₃; U-T5: undisturbed + 32 °C; U-T6: undisturbed + 38 °C; U-T7: undisturbed + cut in half).

Table 12. Average (\pm standard error) marketable and unmarketable plant production (expressed as number and grams of tuber per plant) of three varieties subjected to seven dormancy-breaking treatments in the 2000 growing season (Experiment 2).

Treatments	Marketable Yield						Unmarketable Yield					
	Tuber Number (no. plant ⁻¹)			Tuber Weight (g plant ⁻¹)			Tuber Number (no. plant ⁻¹)			Tuber Weight (g plant ⁻¹)		
	Ari	Mon	Spu	Ari	Mon	Spu	Ari	Mon	Spu	Ari	Mon	Spu
R-T1	4.1 \pm 1.0	4.0 \pm 1.0	2.7 \pm 1.0	578.7 \pm 34	428.9 \pm 41	487.6 \pm 44	0.3 \pm 0.01	0.3 \pm 0.01	0.6 \pm 0.01	31.2 \pm 4.3	25.8 \pm 3.0	102.2 \pm 12
R-T2	5.2 \pm 1.4	4.7 \pm 1.3	3.2 \pm 1.0	586.6 \pm 45	520.1 \pm 49	440.8 \pm 39	0.4 \pm 0.01	0.2 \pm 0.01	0.8 \pm 0.01	24.9 \pm 3.8	6.3 \pm 2.3	113.8 \pm 9
U-T3	4.7 \pm 1.0	3.8 \pm 1.0	3.6 \pm 1.0	628.1 \pm 66	491.7 \pm 37	489.3 \pm 45	0.3 \pm 0.01	0.0 \pm 0.01	0.8 \pm 0.01	2.1 \pm 0.1	0.2 \pm 0.01	105.7 \pm 11
U-T4	4.8 \pm 1.0	5.8 \pm 1.6	5.1 \pm 1.4	605.5 \pm 60	649.3 \pm 59	622.8 \pm 64	0.2 \pm 0.01	4.9 \pm 0.56	1.3 \pm 0.01	4.7 \pm 1.9	11.4 \pm 2.4	102.2 \pm 19
U-T5	4.9 \pm 1.5	2.9 \pm 0.9	5.4 \pm 1.5	618.8 \pm 58	388.6 \pm 38	717.8 \pm 68	0.5 \pm 0.01	0.3 \pm 0.01	1.4 \pm 0.09	63.5 \pm 5.6	9.0 \pm 1.6	114.7 \pm 11
U-T6	5.0 \pm 1.5	2.7 \pm 0.5	4.0 \pm 1.0	618.1 \pm 54	393.2 \pm 29	685.9 \pm 65	0.4 \pm 0.01	0.1 \pm 0.01	1.0 \pm 0.01	38.0 \pm 4.7	31.8 \pm 4.5	103.9 \pm 10
U-T7	4.7 \pm 0.9	3.6 \pm 1.0	3.0 \pm 1.0	521.7 \pm 42	459.1 \pm 46	651.1 \pm 64	0.5 \pm 0.01	0.2 \pm 0.01	0.5 \pm 0.01	9.5 \pm 2.2	3.2 \pm 1.0	104.3 \pm 8
Significance	$p > F$			$p > F$			$p > F$			$p > F$		
Variety (V)	<0.001			0.042			0.552			<0.001		
Treatment (T)	<0.001			0.032			0.031			0.142		
V \times T	<0.001			0.057			0.425			0.119		

Ari, Mon, Spu: Arinda, Monalisa, Spunta varieties, respectively. R-T1: removed + 20 °C; R-T2: removed + 20 °C + GA₃; U-T3: undisturbed + 20 °C; U-T4: undisturbed + 20 °C + GA₃; U-T5: undisturbed + 32 °C; U-T6: undisturbed + 38 °C; U-T7: undisturbed + cut in half.

4. Discussion

In the seed potato sector, long dormancy periods result in a better quality of tuber conservation during storage. However, in the perspective of seed-tuber production, prolonged and non-regular dormancy is often an undesirable condition especially when the objective is to have many generations of tubers or when the health status of the seed-tuber must be certified. Even though the breakage of dormancy has been extensively studied, only a few effective techniques can be found (and considered as easily applicable) in the current scientific literature. The main aim of the study was to improve the techniques used for the breakage of seed-tuber dormancy for offseason potato production. In two different experiments we evaluated seven treatments based on the combination of physical, hormonal, and mechanical factors such as different range of temperature, combination of thermal treatments with gibberellic acid application, cutting in half of tubers, and early haulms removal. Furthermore, the response to treatments aimed to break the dormancy may be highly affected by genotypes. Indeed, potato varieties may be also ranked depending on the depth and duration of the dormancy period. We monitored four varieties, Europa and Arinda tested for one year each, and Spunta and Monalisa present in both experiments. We decided to use varieties that were widespread at national (Monalisa) [30,31] and international (Arinda, Europa and Spunta) [3,30] scale and, at the same time, that were representative as regards the period of dormancy. In fact, Europa and Arinda are characterized by a short dormancy period, whereas Spunta and Monalisa have a medium and long dormancy period, respectively (<http://triskalia-potato.com/our-variety/>).

In this study we confirmed that (i) Monalisa has deeper dormancy than Spunta, Arinda and Europa, and that this difference is unaffected by growing season; (ii) the meteorological weather trend of the growing season influenced the seed-tuber age and thus the response of seed-tubers to the application of breaking-dormancy treatments; and that (iii) while potato varieties, belonging to short- and medium-dormant classes (Arinda, Europa, and Spunta), impacted the sprouting activity (higher for short- and medium-dormant genotypes), the most efficient dormancy release (defined as 80% of 2 mm sprouts) was obtained by applying 20 °C plus GA₃ (Figures 2 and 3).

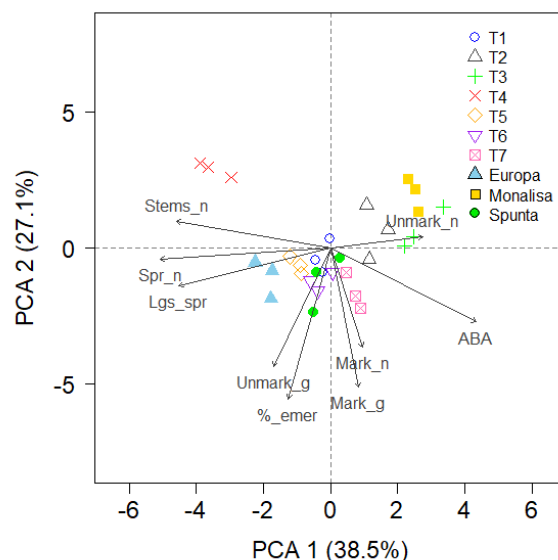


Figure 3. Principal component analysis (PCA) biplot showing patterns and interaction between variables and treatments for Experiment 1. ABA, abscisic acid content; Sprout_n, number of sprouts per tuber; Lgst_sprout, the longest sprout; %_emer, percentage of plant emergence; Mark_g, grams of tuber per plant; Mark_n, number of tuber per plant; Unmark_g and Unmark_n, grams and number of unmarketable tuber per plant, respectively. T1: 20 °C; T2: 10/20 °C; T3: 2/20 °C; T4: 38/20 °C; T5: 20 °C + GA₃; T6: 10/20 °C + GA₃; T7: 2/20 °C + GA₃.

The initial content of ABA was very different between years at the starting-point of experiments. In fact, during the first year the tubers contained on average 15 ng of ABA g⁻¹ fresh weight, whereas during the second year, they contained about 1/3 of ng of ABA g⁻¹ fresh weight (on 30 June). Although, there is no evidence of an ABA threshold concentration for dormancy release [42,43], we hypothesized that in the second year the thermal trend that occurred in open-field condition before the harvest had influenced the dormancy status of tubers. Previous studies reported that dormancy is lost most rapidly when temperatures are warm just before harvest, showing the greatest reduction in dormancy at temperatures greater than 35 °C [8]. Thus, according to previous findings, the lowest ABA content recorded in Exp. 2 at the beginning of the storage phase was probably attributable to the degree of maturity at harvest that influenced physiological age [26]. Degree of maturity in 2000 was significantly accelerated by a warm spring relative to 1999 (Figure 1a). The accumulated growing degree days (base = 2 °C) at 125 days after planting in 2000 was 2447 versus 2069 in 1999.

These results showed that T4 treatment which provided the exposure of seed-tuber at 38/20 °C was the most effective both for dormancy termination and for promoting sprouting, especially for Europa (Exp-1), Arinda (Exp-2), and Spunta. In Exp-1 at 28 days after starting of the experiment, T4 treatment induced a decrease in ABA content about two-fold lower than the remaining treatments, and more than three-fold higher sprouting activity (number of sprouts longer than 2 mm). Seven days after the GA₃ application the dormancy was terminated in T5 (20 °C + GA₃) and T1 (20 °C). Thus, according to Suttle [8] it seems that thermal treatment had the main role in releasing dormancy, more than the application of GA₃ in itself. The same pattern was also observed in the Exp-2 where following the application of thermal treatments, as early as 24 August, and before the application of gibberellic acid, the content in ABA was rather low. Some authors confirmed that gibberellins are involved in induction of sprouting activity, sprout vigor and growth rather than in releasing seed-tubers dormancy [44–46]. The cutting of the seed-tubers in half was shown to be not only a technique aimed at reducing planting costs. As expected, U-T7 treatment (undisturbed + cut in half) resulted in a lower content of ABA and in vigorous sprouting activity. At this regard, Otrushy and Struik [47] explained that the cutting of seed-tuber causes the break of dormancy as a consequence of the breakage of apical dominance.

Early haulm removal is a usual technique in potato growing aimed to avoid aphids attacks [12], to improve tubers set distribution and size, to foster periderm maturation [48], and to affect also physiological aging [12]. Findings of Exp-2 suggested that the early haulms removal did not affect the termination of dormancy, in fact in accordance with the last date of analysis (29 August) the content of ABA in R-treatments was significantly higher than in other treatments irrespective of the use of GA₃. Consistently, Brown et al. [49] did not observe any significant differences among early haulm killing or natural haulm senescence with respect to dormancy duration. The sprouting activity, which is closely connected to the end of dormancy, was evaluated as the number of shoots longer than 2 mm and the longest shoot length in Exp-1 and through the use of a visual scale in Exp-2. Observations on sprouting activity confirmed results on ABA content. In Exp-1, treatment T4 produced 3.5 sprouts per tuber, between 30 August and 6 September sprouting was highly favored by the application of GA₃. Basically, results of Exp-1 related to the sprouting activity were qualitatively similar to the findings of Exp-2, since the main results of the visual evaluation were that treatments with storage at 20 °C, 20 °C + GA₃, and the cutting of the tubers in half resulted in the highest sprouting activity. Data suggest that exogenous GAs are not intimately involved with tuber dormancy control, but that they have a key-role in subsequent sprout elongation [21,50]. The length of the longest sprout has been shown to be one of the most useful measurements to estimate sprout development in potato tubers [51]. It has been observed in previous studies that multiple sprouting cultivars show a slower growth rate of their longest sprout [52]. In the case of Exp-1, irrespective of the variety, the longest sprout resulted in being associated with the highest number of sprouts per tuber longer than 2 mm, thus consistent with Carli et al. [38] that did not find any correlation between number of sprouts and length of the longest sprout. Moreover, as regards genotype, the longest sprout was recorded in Europa and Spunta in accordance with results of Carli et al. who reported that clones with shorter dormancy often showed a

greater length of their longest sprout [38]. Principal component analysis confirmed in particular that sprouting characteristics such as number of sprouts longer than 2 mm, the length of the longest sprout, and sprouting activity, were as affected by dormancy class as by breaking-dormancy treatments, and that probably there was a synergistic effect between the combination short-, medium-dormant varieties and 20 °C + GA₃ (T5, T6 in Exp-1, and U-T4 in Exp-2) breaking-dormancy treatment.

At the open-field scale, it is necessary to point out that treatments based on the combination between thermal range and GA₃ application led to a prompt and vigorous plant emergence, indeed, since the first monitoring date we observed a clear distinction between thermal treatments and thermal + hormonal treatments. Similarly, in the Exp-2, where plant emergence was evaluated at 24 days after planting, plants derived from tubers treated with gibberellic acid showed a quicker emergence, regardless of the mother plants originally removed early before harvest or left undisturbed. These results are consistent with previous studies [23] in which a comparison among different dormancy-breaking treatments led to the conclusion that treatment composition significantly influenced plant emergence and that GA₃ was essential for subsequent growth and emergence of potato plants. In Exp-1, among the studied treatments, T4 (38/20 °C) showed the lowest emergence percentage (only 62%) and crop performance. In this last case, we hypothesized that seed tubers were used late with respect to their physiological age. Indeed, Struik [53] suggested that the cumulative temperature during post-harvest storage is the most important factor affecting physiological aging, although its effect is moderated by genotypes. A physiologically older seed accelerates the growth rhythm of potatoes due to which the yield develops earlier, while the yield formation ability decreases [54,55]. Moreover, other authors stated the physiological age of seed-tubers affects future crop performance [56]. By contrast, during the second year T5 (32 °C) and T6 (38 °C) had crop performances that did not differ from other treatments. The explanation lies in the duration of the exposure time at high temperatures which was 10 days during the Exp-1 and 5 days during the Exp-2 with a consequent lower value of accumulated thermal units. The crop performance of Exp-2 was in line with respect to results of Eremeev et al. [56] that kept seed-tubers at 30 °C for 5 days before planting and found that pre-planting thermal shock treatment increased significantly the number of tubers per plant compared to control. In both experiments, apart from T4 in Exp-1, on average GA₃ treatments showed a higher total tuber weight. Indeed, other authors reported that treatments of seed-tuber with GA also affect tuber set and size [57].

5. Conclusions

Although the two experiments did not fully overlap in terms of treatment composition and varieties, the results achieved were quite comparable. Indeed, thermal treatments alone and combined with GA₃ significantly reduced the dormancy of the potato tubers in both experiments, while in Exp-2 early haulm killing was less important in releasing dormancy. The effectiveness of dormancy-breaking treatments was closely related to the variety since the short-dormant varieties showed a more rapid response.

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