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Carbon disulphide promotes sprouting of potato minitubers

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Abstract

We investigated the effects of postharvest application of carbon disulphide (CS_2) in various concentrations (0, 15, 25, 35, 45 and 55 ml m^{-3}) and with different exposure duration (24, 48, 72 and 96 h) on breaking of dormancy and sprouting of potato (*Solanum tuberosum* L., cv. Marfona) minitubers of two ages (freshly harvested and one week after harvest) and two weight classes (1.5 and 12 g). In comparison with the control minitubers, CS_2 treated minitubers showed significantly shorter dormancy and better sprouting. More rotting and weaker responses were observed in freshly harvested treated minitubers compared with minitubers treated one week after harvest. The number of sprouts per minituber and their length were significantly enhanced by treating minitubers with CS_2 compared with the untreated control minitubers, but there were strong interactions with minituber weight. Results showed that duration of CS_2 treatment was more important than concentration and longer duration of CS_2 treatment exhibited a stronger action on breaking dormancy and sprouting of potato minitubers than shorter treatments. But, when longer duration was accompanied with higher concentration, treatment with CS_2 led to formation of needle sprouts, which are undesirable as they do not produce vigorous stems.

Keywords: concentration; dormancy; exposure duration; needle sprouts; rotting; seed weight

Abbreviations: Con_ concentration; CS_2_carbon disulphide; Dur_ duration of exposure; GA_3_gibberellic acid

Introduction

One of the most popular ways to produce pre-basic seed of potato (*Solanum tuberosum* L.) is to grow minitubers in the greenhouse from *in vitro* plantlets produced from nodal cuttings. Minituber production has found its place in the seed production systems all over the world as it creates a bridge between the *in vitro* rapid multiplication based on nodal cuttings and the field multiplication of seed tubers. Minitubers are more flexible, can be stored and mechanically planted, and show a larger vigour than either microtubers or *in vitro* plantlets (Struik, 2007a). Similar to normal seed tubers, minitubers go through different stages of development after harvest, including dormancy (no sprout), apical dominance (only one sprout), normal sprouting (a few, normal sprouts per seed tuber), advanced sprouting (many sprouts per seed tuber which are often branched), senility (excessive sprouting with very weak sprouts), and incubation (little tuber formation) (Struik, 2007b). The physiological status of seed potatoes has a great impact on the emergence, number of stems per plant, number of tubers per stem, tuber-size distribution, and tuber yield of the progeny crop (Struik et al., 2006). This is also true when minitubers are used (Struik and Lommen, 1999; Struik and Wiersema, 1999). After harvest, normal seed tubers show dormancy for about 1−15 weeks, depending on cultivar, tuber size, conditions before harvest and storage conditions. Small tubers, such as minitubers, even have longer periods of dormancy (Lommen, 1993) and are more sensitive to adverse conditions during storage (Struik and Wiersema, 1999). The progress of the physiological ageing can be accelerated by conditions during storage, especially by storage temperature. Cold shocks, heat shocks and warm temperatures all advance breaking of dormancy (Struik and Wiersema, 1999). But, when the time between harvesting and planting is very short, these methods might not be effective (enough). Chemical dormancy breaking is then an option to achieve rapid and uniform crop emergence as well as a high number of stems per plant. In Iran, a large amount of minitubers is harvested between March and April. Normal planting time of these minitubers in the field or in the greenhouse is in the month of May or beginning of June. So there is not enough time between harvest and planting to break dormancy naturally. This calls for a reliable technique to break the dormancy of these small tubers chemically. At commercial scale, Rindite (Rehman et al., 2001), bromoethane (Coleman, 1983), CS_2 (Meijers, 1972) and GA_3 (Alexopoulos et al., 2008) have been used to break the dormancy of potato seed tubers. There are only a few records about the effect of CS_2 on potato dormancy,

Table 1. Effects of minituber weight and age on the sprouting of potato minitubers

Minituber weight (g)	Minituber age (after harvest)	Days until sprouting	Sprout length (mm)*	Number of sprouts per minituber			Needle sprout	Rotting
				> 5 mm	< 5 mm	Total		
1.5	0 week	44.7 a	7.5 d	0.2 d	1.1 c	1.3 d	-	yes
	1 week	32.0 c	15.8 b	0.4 c	1.2 c	1.7 c	yes	seldom
12	0 week	40.0 b	12.7 c	0.6 b	1.6 b	2.3 b	-	yes
	1 week	26.7 d	28.0 a	1.1 a	1.9 a	3.0 a	yes	seldom
	LSD	1.84	1.70	0.05	0.12	0.13		

* Sprout length was measured only in those minitubers which had sprouts longer than 5 mm

because the dormancy breaking effects of Rindite and bromoethane are considered stronger than the effect of CS_2. However, CS_2 might have some important advantages compared with common commercial treatments. For normal seed tubers, is has been shown that the efficacy of CS_2 at low concentration (12 – 25 ml m^{-3}; Meijers, 1972) offers a more economical method to break potato dormancy in comparison with bromoethane (100 – 200 ml m^{-3}, Coleman, 1983) and Rindite (200 – 400 ml m^{-3}, Kim et al., 1999). This economic advantage is relevant, especially in developing countries. CS_2 might also be safer than some of the other commercial compounds as minitubers may be too delicate to withstand coarse chemical treatment. Moreover, some potato cultivars hardly respond to GA_3 treatment. Also, treating minitubers with GA_3 induces excessive sprout elongation resulting in sprouts that are thin, fragile, and prone to breakage during handling (Suttle, 2008; Salimi et al., 2010). In contrast, treatment with CS_2 effectively terminates dormancy and the resulting sprouts are short, thick, robust, and resistant to breakage (Salimi et al., 2010). Breaking the potato minituber dormancy using optimal application of CS_2 might therefore be beneficial. Hence, in this study we investigate factors influencing the CS_2 efficacy in breaking potato minituber dormancy shortly after their harvest in order to design an optimal protocol for dormancy breaking under the seed system conditions of Iran.

Materials and methods

Minitubers from cultivar Marfona (medium to long natural dormancy) were produced on *in vitro* propagated plantlets by the Pishtaz Tissue Culture Company (Karaj, Iran) in 2008. The *in vitro* propagated plantlets were planted in a seed box, containing a 3:1 (v/v) mixture of peat and perlite, in a greenhouse at a day/night temperature of 20/14 ^{o}C and a day length of 14 h. Minitubers were hand harvested 120 days after planting. Three replications of 10 minitubers of two weight classes (average weight of about 1.5 and 12 g) were treated with carbon disulphide (CS_2) evaporation with a concentration of 15, 25, 35, 45 and 55 ml m^{-3} or not treated (control). For the carbon disulphide (Merck, Germany) application, minitubers were put in 31.6 L plastic containers with tightly fitting lids at room temperature for 24, 48, 72 and 96 h. Sufficient CS_2 was supplied in liquid form in 25 ml beakers to give the required concentration in the container volume. Moreover, we used minitubers of two different ages: zero (i.e. freshly harvested) or one week after harvest. Following treatments, minitubers were air dried and then placed in the dark at 25±0.3ºC and 85±5% RH. Minitubers were considered sprouted when a minituber had at least one sprout with a length of at least 2 mm. The development of sprouts of the minitubers was recorded at two-day intervals until all minitubers had sprouted. The dormant period was assessed as number of days from treatment to sprouting, and was considered to have ended when 80% of the minitubers had at least one sprout with a length of at least 2 mm. After the end of dormancy, presence of needle sprouts and presence of rotted minitubers were evaluated and the average number of sprouts present per minituber and the sprout length were assessed. Sprout length was measured only in those minitubers which had sprouts longer than 5 mm.

Results and discussion

Dormancy period

Minitubers of 1.5 g had a longer dormancy period than minitubers of 12 g (Table 1), an effect which is consistent with literature (Lommen, 1993). Minitubers with an age of one week showed shorter dormancy (Table 1), thus confirming Lommen (1993) and Struik and Wiersema (1999). Irrespective of duration and concentration, the effects of CS_2 treatment on dormancy break were clear throughout the whole experiment: CS_2 treatment consistently advanced sprouting (Tables 2 and 3). However, the dormancy shortening effect of CS_2 treatment was more effective in one-week old minitubers than in freshly harvested minitubers. In addition, in freshly harvested minitubers in which proper skin set had not occurred yet, CS_2 treatment led to rotting and localized necrosis development, especially when duration of the exposure or concentration increased. However, freshly harvested minitubers of 12 g, which were treated with 45 ml m^{-3} for 48 h, showed very uniform responses. All minitubers sprouted within 10 days (data not shown) and showed normal sprouting (data not shown). Therefore this treatment is very suitable to break dormancy. In both weight classes of one week old minitubers, at low concentration, dormancy period decreased with an increase in the duration of treatment, while for high concentrations, short duration was

Table 2. Effects of the duration of the exposure (Dur) and the concentration (Con) of CS_2 on sprouting characteristics of one week old minitubers (weight 1.5 g)

Con × Dur (ml m^{-3}) (h)		Days until sprouting	Final sprout length (mm)	Final number of sprouts per minituber			Needle sprout
				> 5 mm	< 5 mm	Total	
control		70 ± 3.2	5.7 ± 0.7	0.1 ± 0.10	1.1 ± 0.07	1.2 ± 0.10	-
15	24	47 ± 3.3	5.9 ± 0.7	0.1 ± 0.10	1.4 ± 0.43	1.5 ± 0.34	-
	48	42 ± 3.5	9.3 ± 1.4	0.1 ± 0.04	1.3 ± 0.07	1.4 ± 0.04	-
	72	36 ± 3.1	16.6 ± 1.4	0.4 ± 0.14	1.3 ± 0.10	1.7 ± 0.04	-
	96	33 ± 4.6	17.0 ± 1.4	0.5 ± 0.07	1.1 ± 0.32	1.6 ± 0.28	-
25	24	39 ± 3.8	10.0 ± 2.1	0.3 ± 0.07	1.0 ± 0.14	1.3 ± 0.10	-
	48	35 ± 1.8	10.6 ± 1.0	0.3 ± 0.04	1.3 ± 0.10	1.7 ± 0.14	-
	72	34 ± 2.1	17.6 ± 1.7	0.4 ± 0.10	1.2 ± 0.16	1.7 ± 0.07	-
	96	31 ± 2.7	18.0 ± 2.5	0.6 ± 0.07	1.3 ± 0.28	1.9 ± 0.24	-
35	24	37 ± 2.9	8.6 ± 0.8	0.3 ± 0.04	1.0 ± 0.14	1.4 ± 0.16	-
	48	36 ± 3.9	14.6 ± 1.0	0.4 ± 0. 00	1.3 ± 0.21	1.7 ± 0.29	-
	72	35 ± 4.4	18.0 ± 2.1	0.6 ± 0.14	1.3 ± 0.41	1.9 ± 0.31	-
	96	35 ± 0.4	19.3 ± 3.9	0.6 ± 0.07	1.3 ± 0.10	1.9 ± 0.04	yes
45	24	42 ± 4.1	20.3 ± 3.1	0.4 ± 0.04	0.9 ± 0.17	1.3 ± 0.17	-
	48	41 ± 0.8	17.3 ± 2.2	0.6 ± 0.10	0.9 ± 0.28	1.5 ± 0.22	-
	72	32 ± 2.1	19.3 ± 3.3	0.5 ± 0.16	1.1 ± 0.21	1.6 ± 0.26	yes
	96	34 ± 5.6	18.3 ± 2.2	0.5 ± 0.04	1.3 ± 0.10	1.8 ± 0.08	yes
55	24	36 ± 6.7	18.3 ± 2.6	0.6 ± 0.12	0.8 ± 0.14	1.4 ± 0.04	-
	48	33 ± 2.4	15.0 ± 1.4	0.5 ± 0.10	1.3 ± 0.24	1.9 ± 0.14	-
	72	32 ± 5.5	18.6 ± 1.6	0.5 ± 0.12	1.6 ± 0.39	2.1 ± 0.43	yes
	96	35 ± 7.1	23.0 ± 2.1	0.5 ± 0.25	1.5 ± 0.14	2.0 ± 0.12	yes

more effective (Tables 2 and 3). This results suggests that treatment costs will be lowest if minitubers are treated at a low concentration and for a relatively long period.

Final sprout length

Minitubers of 1.5 g had shorter sprouts than minitubers of 12 g (Table 1), an effect which is consistent with an earlier report by Lommen (1994). Minitubers with an age of one week showed longer sprouts than freshly harvested minitubers (Table 1), which is in accordance with Lommen (1994) and Struik and Wiersema (1999). Irrespective of duration and concentration, treating minitubers with CS_2 promoted sprout growth (Tables 2 and 3). In minitubers of 1.5 g, there was a clear effect of duration at the lower concentrations only and the length of sprouts tended to increase with an increase in the duration of CS_2 treatment (Table 2). But, with increasing concentration these differences diminished. Effect of concentration on sprout length was not very obvious. In minitubers of 12 g, there was a clear effect of duration at all concentrations and the length of sprouts tended to increase with an increase in the duration of CS_2 treatment (Table 3). But, in higher concentrations, an exposure of 72 h induced longer sprouts than an exposure of 96 h, most likely due to necrosis in tips of sprouts in the longer exposure. Effects of concentration were also obvious, higher concentration inducing longer sprouts. In case of high concentration, especially when exposure was long, the sprouts became damaged as buds started to grow while exposed to CS_2 evaporation. As a result, the apical sprout died off and necrosis was observed in the tips of lateral sprouts.

Table 3. Effects of the duration of exposure (Dur) and the concentration (Con) of CS_2 on sprouting characteristics of one week old minitubers (weight 12 g)

Con × Dur ($ml\ m^{-3}$) (h)		Days until sprouting	Sprout length (mm)	Final number of sprouts per minituber			Needle sprout
				> 5 mm	< 5 mm	Total	
control		65 ± 2.4	8.9 ± 0.7	0.4 ± 0.10	0.9 ± 0.07	1.3 ± 0.10	-
15	24	38 ± 3.9	16.3 ± 2.8	0.9 ± 0.04	0.6 ± 0.10	1.6 ± 0.10	-
	48	33 ± 3.5	27.3 ± 2.9	1.0 ± 0.07	1.7 ± 0.10	2.7 ± 0.08	-
	72	32 ± 1.0	30.3 ± 1.2	1.0 ± 0.04	2.0 ± 0.24	3.0 ± 0.22	-
	96	33 ± 2.3	33.3 ± 3.6	0.9 ± 0.08	2.4 ± 0.18	3.3 ± 0.10	yes
25	24	36 ± 2.2	16.3 ± 4.2	0.8 ± 0.2	0.8 ± 0.18	1.6 ± 0.10	-
	48	26 ± 2.6	27.0 ± 4.2	1.0 ± 0.04	1.6 ± 0.10	2.7 ± 0.07	-
	72	24 ± 2.4	33.6 ± 3.6	1.1 ± 0.04	2.2 ± 0.37	3.3 ± 0.34	-
	96	23 ± 4.0	39.6 ± 2.8	1.0 ± 0.04	2.9 ± 0.51	3.9 ± 0.53	yes
35	24	35 ± 1.7	17.3 ± 2.8	0.9 ± 0.04	0.7 ± 0.10	1.7 ± 0.08	-
	48	35 ± 1.0	29.6 ± 4.7	1.1 ± 0.04	1.8 ± 0.07	2.9 ± 0.08	-
	72	24 ± 3.1	34.0 ± 0.8	1.4± 0.07	2.3 ± 0.37	3.8 ± 0.43	-
	96	33 ± 5.6	30.6 ± 5.3	1.0 ± 0.04	2.0 ± 0.24	3.0 ± 0.20	yes
45	24	35 ± 3.9	23.3 ± 2.2	0.7 ± 0.18	1.6 ± 0.40	2.3 ± 0.40	-
	48	35 ± 2.1	35.0 ± 1.6	1.2 ± 0.04	2.2 ± 0.10	3.5 ± 0.07	-
	72	22 ± 1.4	39.6 ± 2.0	1.6 ± 0.22	2.3 ± 0.21	3.9 ± 0.42	yes
	96	34 ± 5.5	35.0 ± 5.7	1.0 ± 0.14	2.5 ± 0.39	3.5 ± 0.53	yes
55	24	32 ± 2.1	25.0 ± 3.6	0.9 ± 0.07	1.6 ± 0.44	2.5 ± 0.49	-
	48	25 ± 3.1	30.6 ± 0.8	1.4 ± 0.12	1.6 ± 0.65	3.0 ± 0.73	-
	72	30 ± 3.2	41.3 ± 0.8	1.5 ± 0.07	2.6 ± 0.21	4.1 ± 0.14	yes
	96	36 ± 1.4	38.6 ± 3.7	1.0 ± 0.04	2.8 ± 0.00	3.8 ± 0.04	yes

Number of sprouts per minituber

Number of large sprouts

Minitubers of 1.5 g had fewer large sprouts per minituber than minitubers of 12 g (Table 1), whereas minitubers with an age of one week showed more large sprouts per minituber than freshly harvested minitubers (Table 1). In general, treating minitubers with CS_2 promoted the number of large sprouts per minituber (Tables 2 and 3). In minitubers of 1.5 g, there was a clear effect of duration of exposure at the lower concentrations and the number of large sprouts per minituber tended to increase with an increase in the duration of CS_2 treatment. But, in minitubers of 12 g, a similar effect of duration of exposure at higher concentration was observed with the difference that the number of large sprouts was reduced again at 96 h exposure. Effects of concentration were not clear.

Number of small sprout

Minitubers of 1.5 g had fewer small sprouts per minituber than minitubers of 12 g (Table 1). Minitubers with an age of one week showed more small sprouts per minituber than freshly harvested minitubers (Table 1), but only so in the larger size class (Table 1). In general, treating minitubers with CS_2 increased the number of small sprouts per minituber (Tables 2 and 3). In minitubers of 1.5 g, there was a clear effect of duration of exposure at the higher concentrations and the number of small sprouts per minituber tended to increase with an increase in the duration of CS_2 treatment. But, in minitubers of 12 g, there was a clear effect of duration of exposure at all concentrations and the number of small sprouts tended to increase with an increase in the duration of CS_2 treatment. Meijers (1972) stated that a small overdose results in dying off of the very young apical sprout(s), but following an initially delayed sprouting, as many or even more sprouts then develop on giving an optimal dose.

Total number of sprouts

Minitubers of 1.5 g had fewer sprouts per minituber than minitubers of 12 g (Table 1), which confirms an earlier report by Lommen (1994). Minitubers with an age of one week showed more sprouts per minituber than freshly harvested minitubers (Table 1), in agreement with Lommen (1994) and Struik and Wiersema (1999). Irrespective of duration and concentration, treating minitubers with CS_2 promoted the total number of sprouts per minituber (Tables 2 and 3). In minitubers of 1.5 g, there was a clear effect of duration of exposure on total number of sprouts at the highest concentrations only and the number increased with an increase in duration of exposure. But at low concentrations longer exposure caused an increase in the proportion of large sprouts. Thus the increase in duration is important in identifying the best possible treatment.

Needle sprouts

Formation of needle sprouts can be induced when higher concentrations were applied, especially with longer duration of exposure. Effects were most pronounced in minitubers of 12 g.

Results have shown that to break dormancy and to produce long sprouts a long treatment with a low dose is best. After such a treatment, minitubers can be planted that are no longer dormant but also have a good vigour. Lommen (1994) reported that if minitubers were planted with longer sprouts the time to emergence could be shortened and differences between minitubers of different weights could be reduced. In minitubers of 12 g, there was a clear effect of duration of exposure at all concentrations with a possible optimum at the higher concentrations. In minitubers of 12 g, after CS_2 treatment in one week after harvest a large number of eyes (sometimes all eyes) produced sprouts. In such a case, minitubers would not contain enough substrates and nutrients to serve all sprouts equally well and competition among sprout would increase. In addition, the high number of sprouts would increase respiration and evaporation from minitubers before planting and this might deplete the resources of the minituber. Also, some of these sprouts (the needle sprouts and those with tip necrosis) will not produce a viable stem and can perhaps even temporarily parasitize on other sprouts of the same minituber or at least on the mother tuber. As a result, planting of minitubers with many sprouts may lead to reduced growth vigour of individual stems (Struik and Wiersema, 1999).

Conclusions

The dormancy period decreased with an increase in the duration of the treatment, but concentration had little effect. However, to determine the best concentration and duration for CS_2 treatment, the sprouting quality (length and number of sprouts) of minitubers was an important determinant and this quality was significantly influenced by minituber weight class. Our results demonstrate that CS_2 effectively shortened dormancy especially when minitubers were treated one week after harvest. Treatments of small minitubers with low concentration and long duration (e.g. 25 ml m^{-3} for 96 h) had an optimum effect on breaking of dormancy and on sprouting. But in large minitubers, an increase in duration of exposure to CS_2 led to such an increase in sprout number that sprouts would not have enough growth vigour when these minitubers would be planted in the field or in the greenhouse. Treating large minitubers with shorter duration (35 ml m^{-3} for 48 h in one week old minitubers or 45 ml m^{-3} for 48 h in freshly harvested minitubers), would provide suitable sprouting although the effect on breaking of dormancy might be slightly less than with longer exposure. Therefore, the results of present study could be used for commercial seed tuber producers to provide farmers with minitubers having no level of tuber dormancy and reduce the risk of stand establishment in the farm. Likewise, study of the physiological and molecular basis of these dormancy breaking treatments in future will be important.

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