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Parthenocarpic fruit set in triploid watermelon induced by CPPU and 2,4-D applications

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Abstract

The localized application of the synthetic cytokinin CPPU ((2-chloro-4-pyridyl)-*N*-phenyl urea) to ovaries at flower opening was as effective as free pollination in setting parthenocarpic fruit in the triploid watermelon cultivar 'Reina de Corazones', and increased yield per unit land area by at least 50%, simply due to the lack of requirement for diploid pollen producing plants within the orchard. The application of the synthetic auxin 2,4-D (2,4-dichlorophenoxy acetic acid) as a full coverage spray, was also effective in setting fruit; total yield was however 10% smaller than in the CPPU-treated plots, but the cost of application was much less expensive. These applications had no adverse effect on fruit quality, and their effectiveness in commercial watermelon production was evaluated over 4 years. Localized applications of 2,4-D to ovaries were less effective in setting fruit, and increased hollow fruit.

Introduction

European consumers show a marked preference for seedless watermelons. For this reason, the use of triploid cultivars has increased markedly, and nowadays they are significant proportion (50%) of the total watermelon production in Spain. The triploid watermelon cultivars show stimulative parthenocarpy, setting seedless fruit when pollinated with viable and compatible pollen (Maynard 1989). To this aim, diploid plants are intercropped among triploids to serve as a source of pollen. To ensure effective pollination, it is considered necessary to plant one diploid for every 2 triploids (Miguel and Maroto 2000). The use of natural pollinators to set fruit does however pose problems. Temperatures during flowering are often too low for bee activity, resulting in limited pollination (Maynard 1989; Hayata et al. 1995). It would also be considerable advantage to not waste acreage on diploid pollinators, which produce commercially worthless fruit. For these reasons, the set of fruit through the application of growth regulators seems preferable.

There are reports on the use of growth regulators to induce fruit set in watermelon using both synthetic cytokinins and auxins. A localized application of the synthetic cytokinin CPPU, to ovaries, set parthenocarpic fruit both in diploid (Hayata et al. 1995) and triploid watermelon (López-Galarza et al. 2004). However similar application of the auxins IAA (Terada and

Masuda 1940; Miyazaki 1965), NAA or 4-CPA (Kondou and Murozono 1975) to ovaries resulted in insufficient fruit set, a small fruit size and an increase in rind thickness (Primo and Cuñat 1968).

The present work compares the effects of CPPU and 2,4-D on fruit set and quality in the triploid watermelon cultivar 'Reina de Corazones' relative to that achieved through natural pollination. The 2,4-D applications were performed either localized to the ovary, or as a full coverage spray.

Materials and methods

The experiments were performed in watermelon (*Citrullus lanatus* (Thun.) Matsum. and Nakai) producing area in south Valencia, Spain, over a period of 4 years (1997–2000), using the triploid cultivar 'Reina de Corazones', grafted on the hybrid Shintoza (*Cucurbita mixta* × *Cucurbita moschata*). Plants were raised as described by Miguel et al. (2004). Grafted plants were transplanted to the field by mid April at a density of 3500 plants per hectare, and were protected initially by a low polyethylene tunnel. Shortly after transplanting, the plants were topped to force the growth of three to four lateral vines. Fertilizer application and pest control followed standard commercial practices (Maroto et al. 1996).

Two growth regulators CPPU ((2-chloro-4-pyridyl)-*N*-phenyl urea; Kyowa Hakko Kogyo Co., Tokyo), and ethanol amine salt of the auxin 2,4-D (2,4-dichlorophenoxy acetic acid; Antidrop®, Agrodán, Madrid), were used. The non-ionic wetting agent nonylphenyl polyethylene glycol ether was added at 0.01% (v/v) to growth regulator solutions. Localized CPPU and 2,4-D applications were performed by spraying ovaries at flower opening, using a hand sprayer at a rate of 15 l ha^{-1} (1 ml per ovary). These applications were performed on 5–7 flowers per plant. As all flowers did not open the same day, repeated applications to the same plants were necessary. The full coverage 2,4-D spray was performed on a single occasion when there were at least 4 female flowers open per plant, with a spray boom tractor at a rate of 500 l ha^{-1}.

The optimal concentrations for these growth regulator applications, were determined during the first year. Over the next 3 years we compared the effectiveness of these applications at their optimal concentrations, i.e. 100 mg l^{-1} of CPPU and 50 mg l^{-1} of 2,4-D when applied localized to the ovary and 8 mg l^{-1} of 2,4-D in full-coverage sprays. Main crop plants were open pollinated with pollen of the diploid cultivar 'Dulce Maravilla'; these served as controls. The layout of the experiments was a randomized block design, with 4 replicates per treatment and at least 10 plants per replicate.

Selective harvests were performed beginning in June based on commercial ripeness (Maroto 2002). Total yield was recorded and the mean fruit size was calculated. All the fruits were inspected for hollow heart and malformations. Three fruits per replicate were sampled to measure total soluble solids using a refractometer (Atago PR32).

The significance of the differences was assessed by means of an analysis of variance (SAS Institute 1989). The separation of the means was performed according to the lowest significance difference test at $p \leq 0.05$.

Results

The effectiveness of the hormone applications on set fruit was maximal at flowering (data not shown). The response to localized CPPU applications saturated at 100 mg l^{-1} (Table 1). At this concentration, CPPU application had no significant effect on fruit size, fruit sugar concentration or the incidence of hollow fruit, relative to the open pollinated controls. Increasing the concentration of CPPU did not further increase yield or fruit quality, but reduced significantly ($p \leq 0.05$) hollow fruit.

The localized application of 2,4-D to ovaries induced some set (6.6–9.2 kg per plant), but also increased markedly hollow fruit in a concentration-dependent way (Table 2).

A full coverage spray application of 2,4-D solution caused a transitory reduction in leaf growth, accompanied by a curling of young leaves which lasted for about 7 days. Fruit set was maximal over a concentration range of 6–10 mg l^{-1} . In this experiment, the 2,4-D sprays (at 6–8 mg l^{-1}) reduced fruit size and had no effect on total soluble solids or the proportion of hollow fruit (Table 3).

A comparison of the hormone applications made over three further years is shown in Table 4.

Table 1. The effect of a localized CPPU application to the ovaries on yield and fruit quality of watermelon (*Citrullus lanatus* (Thun.) Matsum. and Nakai).

Fruit setting and CPPU concentration	Fruit yield		Fruit size (kg fruit^{-1})	TSS (°Brix)	Hollow fruit (%)
	kg m^{-2}	kg plant^{-1}			
CPPU-treated					
50 mg l^{-1}	6.3 a	17.0 a	3.7	12.4	11.0 c
100 mg l^{-1}	9.0 c	24.3 b	4.0	11.7	8.2 b
200 mg l^{-1}	8.6 c	23.1 b	4.2	11.6	6.0 a
Open pollinated (control)	7.0 b	28.3 c	4.1	11.5	7.2 b
Significance (*F* value)	$p \leq 0.01$	$p \leq 0.01$	NS	NS	$p \leq 0.01$

Open pollinated plants served as controls. Experiment performed during 1997. Different letters in the same column indicate significant differences at $p \leq 0.05$ using LSD test. NS – not significant.

Table 2. The effect of a localized 2,4-D application to the ovaries on yield and fruit quality of watermelon (*Citrullus lanatus* (Thun.) Matsum. and Nakai).

Fruit setting and 2,4-D concentration	Fruit yield		Fruit size (kg fruit^{-1})	TSS (°Brix)	Hollow fruit (%)
	kg m^{-2}	kg plant^{-1}			
2,4-D (localized)					
50 mg l^{-1}	2.5 a	6.6 a	3.9 a	11.9	77.0 b
200 mg l^{-1}	3.4 b	9.2 b	4.7 b	11.6	93.0 c
Open pollinated (control)	7.0 c	28.3 c	4.1 a	11.5	7.2 a
Significance (*F* value)	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.05$	NS	$p \leq 0.01$

Open pollinated plants served as controls. Experiment performed during 1997. Different letters in the same column indicate significant differences at $p \leq 0.05$ using LSD test. NS – not significant.

Table 3. The effect of a full-coverage 2,4-D full spray application on yield and fruit quality of watermelon (*Citrullus lanatus* (Thun.) Matsum. and Nakai).

Fruit setting and 2,4-D concentration	Fruit yield		Fruit size (kg fruit^{-1})	TSS (°Brix)	Hollow fruit (%)
	kg m^{-2}	kg plant^{-1}			
2,4-D (full spray)					
4 mg l^{-1}	7.6 b	20.5 a	5.9 bc	11.9	2.1 a
6 mg l^{-1}	9.0 d	24.3 b	5.7 b	12.7	1.8 a
8 mg l^{-1}	8.3 c	22.4 ab	5.7 b	12.1	3.1 ab
10 mg l^{-1}	8.8 cd	23.8 b	4.6 a	12.0	8.2 c
Open pollinated (control)	6.9 a	27.8 bc	6.4 c	12.1	3.5 ab
Significance (*F* values)	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$	NS	$p \leq 0.01$

Open pollinated plants served as controls. Experiment performed during 1999. Different letters in the same column indicate significant differences at $P \leq 0.05$ using LSD test. NS – not significant.

CPPU proved as effective as pollination in setting fruit. Significant differences in fruit yield per plant between CPPU-treated and the open pollinated fruit were observed in 1997 (higher in open pollinated plants; Table 1) and in 1998 (higher in CPPU-treated ovaries; Table 4). However, the mean value for the four experiments was very similar with both treatments (26.6 and 26.9 kg per plant, respectively). Fruit yield per plant was on average about 10% smaller ($p \leq 0.05$; Table 4) for the 2,4-D full spray application compared to open pollination (the average for the 4 years was 24.2 and 26.9 kg per plant, respectively). Only in the year 2000, was yield per plant higher in the 2,4-D

Table 4. The comparative effectiveness of four fruit setting approaches on yield and fruit quality of watermelon (*Citrullus lanatus* (Thun.) Matsum. and Nakai).

Year and fruit setting procedure	Fruit yield		Fruit size (kg fruit^{-1})	TSS (°Brix)	Hollow fruit (%)
	kg m^{-2}	kg plant^{-1}			
1998 Experiment					
CPPU (localized; 100 mg l^{-1})	12.1 d	32.7 c	4.0 a	11.2	5.5 a
2,4-D (localized; 50 mg l^{-1})	4.3 a	11.6 a	3.8 a	11.0	33.5 c
2,4-D (full spray; 8 mg l^{-1})	9.4 c	25.4 b	5.1 b	11.5	19.2 b
Open pollinated (control)	7.3 b	29.5 c	3.8 a	11.3	6.2 a
1999 Experiment					
CPPU (localized; 100 mg l^{-1})	10.4 c	28.1 b	6.7 b	11.8	0.0 a
2,4-D (localized; 50 mg l^{-1})	7.1 a	19.2 a	4.6 a	12.0	8.2 c
2,4-D (full spray; 8 mg l^{-1})	8.3 b	22.4 a	5.7 b	12.1	3.1 b
Open pollinated (control)	6.9 a	27.8 b	6.4 b	12.1	3.5 b
2000 Experiment					
CPPU (localized; 100 mg l^{-1})	7.9 c	21.4 b	5.7 b	11.9	0.0 a
2,4-D (localized; 50 mg l^{-1})	2.2 a	5.9 a	4.4 a	12.2	8.2 b
2,4-D (full spray; 8 mg l^{-1})	9.8 d	26.5 c	5.7 b	11.8	0.0 a
Open pollinated (control)	5.4 b	21.9 b	5.2 b	12.0	0.0 a
Analysis of variance					
Parameter (degrees of freedom)	Percentage of the total sum of squares				
Year (n = 2)	11.5**	13.3**	86.1**	32.4**	50.5**
Setting procedure (n = 3)	69.5**	68.2**	3.5**	2.2	40.5**
Year × setting procedure	17.4**	16.5**	7.0**	3.7	8.1**
Residual	1.6	2.0	3.4	61.7	0.9
Standard deviation	1.29	1.28	0.35	0.54	0.02

Hormone applications were localized to the ovaries (100 mg l^{-1} of CPPU or 50 mg l^{-1} of 2,4-D) or applied as a full spray to the whole plant (8 mg l^{-1} of 2,4-D). Open pollinated plants served as controls. Results from three consecutive years (1998–2000). For every year, different letters in the same column indicate significant differences at $p \leq 0.05$. **Significant at $p \leq 0.01$

sprayed plants than in those open pollinated ($p \leq 0.05$; Table 4). The localized application of 2,4-D proved far less efficient. In all experiments, this application resulted in a lower yield than open pollination (Table 4).

Yield per unit of land area was higher ($p \leq 0.01$) in CPPU-treated plots (10.1 kg m^{-2}) compared to those in open pollinated (6.7 kg m^{-2}), where 1/3 of land area was occupied by pollinators. The 2,4-D sprayed plots had a yield of 9.2 kg m^{-2}.

The quality parameters measured (fruit size, total soluble solids and hollow fruit) showed marked yearly variations (Table 4), which alone accounted for much of the variability observed (32–86% of the total sum of squares in the analysis of variance; Table 4). Average fruit size over the 4 years of experiments was similar in CPPU-treated and 2,4-D-sprayed plants, but the 2,4-D sprays showed an increase in fruit size in 1998 ($p \leq 0.05$; Table 4), and a reduction in 1997 ($p \leq 0.01$; Table 3), as compared to the open pollinated control. These two hormone treatments had no effect on total soluble solids or hollow fruit, except for the small, albeit significant ($p \leq 0.05$), increase in hollow fruit in the 2,4-D sprayed plants in 1998 (Table 4).

Discussion

In agreement with previous work on auxin treatments (see Introduction) we show the localized 2,4-D application to ovaries was the least effective in inducing fruit set. On the other hand, both localized CPPU applications and 2,4-D sprays were as effective as open pollination in increasing fruit set, while having no significantly adverse effects on fruit quality.

The decrease in TSS concentration reported in muskmelon (Hayata et al. 2000) and in greenhouse-grown watermelon (López-Galarza et al. 2004) as a result of CPPU applications, did no occur in our experiments, possibly because we used a higher concentration of this growth regulator (100 mg l^{-1}). In agreement with this explanation, Hayata et al. (1995) reported that a low CPPU concentration (20 mg l^{-1}) reduced the TSS concentration compared to open pollinated fruit and higher CPPU concentrations. The increase in fruit size and in rind thickness reported by Kano (2000) in 'Kansen' watermelon set with CPPU application was not found in our work. This may either be a cultivar specific habit or result from the very low fruit load (2 fruits per plant) in Kano's experiment (2000). Fruit pigmentation, a key parameter of quality, was not affected by CPPU (data not shown). The limitations in the use of CPPU come from the labour inputs needed to apply the chemical and its price.

To our knowledge this is the first report on fruit set in watermelon by a 2,4-D full spray field application. This technique has definite advantages; 2,4-D is the cheapest of the auxin growth regulators, while the full spray application require less than 1 h per hectare, compared with 24–48 h for the localized CPPU application. The lower cost of full spray application compensates for the 10% reduction in yield per plant compared to the CPPU-treated.

In conclusion, in our experiments both the CPPU applications and the 2,4-D sprays set fruit reliably, and yielded more fruit per unit of land area than open pollination, as land for pollinators is not required, without having adverse effects in fruit quality. The choice between these two hormone application depends primarily on economics, i.e. labour cost.

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