Short communication

Response of strawberry plants to the application of brassinosteroid under field conditions

Respuesta de plantas de frutilla a la aplicación de brasinoesteroides bajo condiciones de campo

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Abstract

Brassinosteroids are steroidal compounds which are required to plant growth and development and they are, also, implied in plant response to abiotic stress. Recently, important advances in the knowledge of brassinosteroids metabolism and signalling were made, both in model plants and in economically important crops. The aim of this study was to evaluate the agronomic response of strawberry plants to the application of Brassinosteroid as a biotechnological alternative for increased strawberry fruit yield at field conditions. Plants of strawberry cv. ‘Florida Festival’, were used in two annual production cycle (2014-2015) in Tucumán, Argentina. Brassinosteroid treatments increased strawberry fruit yield between 9% and 34%, mainly of marketable type fruits. No statistical difference in non- marketable fruit yield among treatments was observed.

Keywords: *Fragaria ananassa*; Brassinosteroid; Plant growth; Steroidal hormones.

Resumen

Los brasinoesteroides son compuestos esteroidales que se requieren para el crecimiento y desarrollo normal de las plantas, pero que además están implicados en la respuesta de las plantas a estrés abióticos. Recientemente fueron realizados importantes avances en el conocimiento del metabolismo y señalización de los brasinoesteroides, tanto en plantas modelos como en cultivos de importancia económica. El objetivo de este estudio fue evaluar la respuesta agronómica de plantas de frutilla a la aplicación de brasinoesteroides como una alternativa biotecnológica para aumentar el rendimiento de la fruta bajo condiciones de campo. Fueron utilizadas plantas de frutilla cv. ‘Florida Festival’, en dos ciclos anuales de producción (2014-2015) en la provincia de Tucumán, Argentina. Los tratamientos con brasinoesteroides aumentaron el rendimiento de la fruta entre 9 % y 34 %, principalmente de tipo comercial. No se observó diferencias estadísticas en frutos descarte (no comerciales) entre tratamientos.

Palabras clave: *Fragaria ananassa*; Brasinoesteroides; Crecimiento vegetal; Hormonas esteroidales.

In plants, the presence of steroidal hormones is a comparatively new finding, since it was not until the seventies of the XXth century that plant steroids compounds with hormonal function were reported, corresponding to the discovery of “*brasins*” extracted from rapeseed (*Brassica napus*) the first mention. In a bean second internode assay, the “*brasins*” (by the time defined as “crude lipid extract”) caused an increase in growth rate (Mitchell *et al.*, 1970). These authors demonstrated that the exogenous application of brassinolide (BL) induces a remarkable growth promotion in the bean second internode assay (Grove *et al.*, 1979).

Brassinolide and related compounds were named brassinosteroids (BRs). They are considered as a new class of plant hormone with wide-ranging biological activity. Recently, important advances in the knowledge of brassinosteroids metabolism and signalling were made, both in economically important crops (Lieselotte *et al.*, 2014).

BRs control events of agronomical relevance

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like plant growth and architecture, photosynthesis and flower set. Besides, BRs could increase plant tolerance/resistance to a wide range of stresses caused by drought, flooding, salinity, extreme temperatures, heavy metals, oxidative stress, and pathogens (Bajguz and Piotrowska-Niczyporuk, 2014). Vriet *et al.* (2012) also reported that altering endogenous BRs levels improve quality of crops.

In such a way, the exogenous application and/or the manipulation of the endogenous BRs content might result in yield and stress tolerance increase in crops, and both approaches have been employed in plant research and agriculture. Thus far, several BRs have been evaluated in the field and have produced significant yield increase in diverse crops. Among these, the application the BRs has increased yields in tomato (Núñez *et al*., 1995), potato (Torres and Núñez, 1997), cactus pear (Cortes *et al*., 2003) and yellow passion fruit (Gomes *et al*., 2006).

Strawberries are cultivated in different parts of the world, including tropical, subtropical, and temperate areas. Argentina produces strawberry during the 12 months of the year. Tucumán province is one of the most important producers (Kirschbaum and Hancock, 2000; Rodríguez *et al*., 2009). During harvest season, berries are classified as marketable and non-marketable fruits, from a fresh market prospective. Non marketable fruits are produced throughout the harvest season, reaching values of 17% of total yields in short day cultivars (Albregts and Chandler, 1993). Generally, berries are discarded because of small size, pest and disease damage, albinism and misshapen. If non marketable fruit occurs frequently, losses may be severe for the grower (Garren, 1981).

Therefore, the aim of this study was to evaluate the agronomic response of strawberry plants (*Fragaria ananassa* Duch.) to application of brassinosteroid as a biotechnological alternative for increased strawberry fruit yield at field conditions. To accomplish this objective, an experiment using strawberry plants of cv. 'Florida Festival' (short day cultivar) was carried out under field conditions. Fruits of strawberry ‘Florida Festival’ have a very firm texture and excellent flavor. Plants were purchased from commercial nurseries at San Carlos, Mendoza, Argentina. Experiments were conducted on a silt loam soil (pH = 5.7; EC = 0.6 mS cm^{-1}), during two annual production cycles (2014-2015) at INTA’s Estación Experimental Agropecuaria Famaillá (27° 03 S, 65° 25 W, 363 m elevation) in Tucumán, Argentina. Planting ridges consisted of raised beds 1.25 m apart, 0.40 m high, 0.50 m wide, covered with black polyethylene mulch, with two rows of plants (50,000 plants ha^{-1}). A rate of 120 kg ha^{-1} of 15-15-15 (nitrogen, phosphorus, potassium) fertilizer was applied as pre-planting fertilizer. The experimental design was in complete randomized blocks, with three replications of 40 plants each. Fruit were harvested from May through October, two or three times a week, according to fruit maturity.

In this work, were used 24 epibrasinolide (EP 24) and DI 31, a spirostanic analogue of brassinosteroid synthetized by the Laboratory of Natural Products at the La Habana University, Cuba. DI-31 [(25R)-3β, 5α-dihydroxyspirostan- 6-one] which global formula is: $C_{27}O_5H_{42}$, is the active principle of BB16 (BIOBRAS 16), commercial formulation. Treatments were carried out 14 days after transferring the plants to field conditions, with the BB16 and EP24 at 0.1 μM (foliar spray), respectively.

Fruits were graded into marketable (> 10 g per fruit) and non-marketable (< 10 g, either with disease symptoms or deformed). The threshold value for marketable fruit was 10 g since fruits over this weight are sold, either for fresh consumption (larger fruit sizes) or processing (smaller fruit sizes). Variables measured were total fruit-yield (kg ha^{-1}) and marketable fruit-yield (kg ha^{-1}). One-way ANOVA analysis and Tukey´s multiple comparison tests ($p < 0.05$) were run using Statistix (Analytical Software, 1996). The marketable and total fruit yield of strawberry treatment with a brassinosteroid (BB16 and EP24) are shown in Table 2 and Table 1.

Table 1. Total yield for different treatments with brassinosteroids in Famaillá (Argentina), 2014 and 2015.

Treatment	Total yield (kg ha^{-1})	
	2014	2015
Control	19,800 b	17,175 b
BB16	21,600 a	23,016 a
EP24	21,050 ab	18,616 b

Means in columns followed by the same letter are not significantly different ($p > 0.05$).

Treatments with brassinosteroids increased strawberry fruit yield, mainly of marketable type. This was observed at values between 9% and 34% higher than non-treated plants. Besides, this represents about 1,800 kg and 5,800 kg of fruit per hectare. Considering that a base fertilization was applied in every treatment when the crop was implanted, we can infer that the brassinosteroid analogue BB16 used conferred some differential advantages to the plants by increasing fruit weight respect to non-treated plants. Probably, this enables them to support a better fruit yield.

Table 2. Marketable yield and fruit number per plant from different treatments with brassinosteroids in Famaillá (Argentina), 2014 and 2015.

Treatment	Marketable yield (kg ha^{-1})		Marketable fruit number per plant	
	2014	2015	2014	2015
Control	17,750 b	13,481 b	20.437 b	16.151 b
BB16	18,900 a	18,581 a	22.534 a	22.427 a
EP24	18,250 b	14,980 b	21.747 ab	17.181 b

Means in columns followed by the same letter are not significantly different (p > 0.05).

Total production of treated plants with a brassinosteroid analogue BB16 was 9% higher than non-treated plants in 2014 and 34% higher in 2015, with values statistically different ($p < 0.05$). However, no statistical difference in non-marketable fruit from different treatment with brasinoesteroids in 2014 and 2015 (Table 3).

Table 3. Non-marketable fruit from different treatments with brasinoesteroids in Famaillá (Argentina), 2014 and 2015.

Treatment	Fruit rule out yield (kg ha^{-1})		
	Control	BB16	EP24
2014	3,821 a	4,011 a	4,069 a
2015	3,694 a	4,435 a	3,635 a
Treatment	Number of small fruits per plant		
	Control	BB16	EP24
2014	7.29 a	7.02 a	7.55 a
2015	8.34 ab	9.82 a	7.79 b
Treatment	Number of fruits deform per plant		
	Control	BB16	EP24
2014	0.08 a	0.07 a	0.05 a
2015	0.04 a	0.13 a	0.42 a
Treatment	Number of sick fruits per plant		
	Control	BB16	EP24
2014	1.30 a	1.55 a	1.38 a
2015	1.21 a	1.50 a	1.09 a

Mean in rows followed by the same letter are not significantly different (p > 0.05).

Total fruit production was improved when cv. 'Florida Festival' was treated with the analogue BB16, but lower values were detected when using the analogue EP24, compared with the control treatment in 2014 and 2015. In 2014 and 2015, the application with the analogue BB16 increased total fruit yield (Table 1) and also marketable fruit yield (Table 2), with significant differences at p < 0.05.

Plants treated with a brassinosteroid analogue BB16 had the highest total yields, showing significant differences with the rest of the treatments (Tables 1, 2). 'Florida Festival' treatment with a brassinosteroid analogue BB16 was also the cultivar that showed the largest number of non-marketable fruit (8-10 fruits/plant) (Table 3). The control treatment was plants with the lowest yield and number of non-marketable fruit (Table 3).

It is known that application of brassinosteroid have great potential to boost crop yield (Vriet *et al.*, 2012). Synthetic BR analogs have been applied to different plant species in attempts to boost yield, but the underlying molecular mechanisms behind observed changes are not completely understood yet. This would require dividing yield into many compounds and analyzing BRs effects. In rice, for example, yield components are divided into plant density, panicle number per plant, grain number per panicle, and average grain weight (Vriet *et al.*, 2012). Also, this increase in the weight of plants respect to salinity may be due to the positive effect of brassinosteroids on various osmolytes in salinized plants, thereby increasing tolerance to salt stress (Zeng *et al.*, 2010; Shahid *et al.*, 2014). Serna *et al.* (2015), showed that application of BB16 decreased the negative effect of salinity on fresh weight of lettuce plants and totally or partially prevented reduction in weight, without affecting the lettuce phenotype. Similar results have been reported in *Arabidopsis thaliana*, rice, chickpea, mustard, wheat, sorghum, and cowpea (Anuradha and Rao, 2003; Vardhini and Rao, 2003; Ali *et al.*, 2007; Hayat *et al.*, 2007; Zeng *et al.*, 2010; Eleiwa *et al.*, 2011; El-Mashad and Mohamed, 2012).

The beneficial effect of the application of brassinosteroid on productivity of crops has been widely described in different crops since they are involved in many functions related to the development of plants, metabolism, signalling and tolerance/resistance to a wide range of stresses and pathogens (Bajguz and Piotrowska-Niczyporuk, 2014; Coll *et al.*, 2015).

Regarding strawberry crop, it was reported the role of BRs in fruit ripening, suggesting the involvement in early fruit development (Chai *et al.*, 2013). Also, it was reported the capacity of BRs in disease response (Albrecht *et al.*, 2012; Belkhadir *et al.*, 2012; De Vleesschauwer *et al.*, 2012; Nahar *et al.*, 2013).

Considering that BB16 and EP24 increased total fruit and, particularly, marketable fruit yield, the aplicattion of BRs is a promising biotechnological alternative for increased fruit yield at field conditions.

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