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Short communication

Benzyladenine treatment promotes floral feminization and fruiting in a promising oilseed crop *Plukenetia volubilis*

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ABSTRACT

Plukenetia volubilis, whose seeds contain a high content of polyunsaturated fatty acids, produces approximately 60 male flowers but only 1–2 female flowers per inflorescence. Increasing the number of female flowers is critical for yield improvement of *P. volubilis*. In this study, we determined the effect of the plant growth regulator 6-benzyladenine (BA) on floral sex determination in *P. volubilis*. Exogenous application of BA converted male flowers on most of the inflorescences to female flowers, and approximately 8–20% of the induced female flowers further developed into fruits. Treatment with various concentrations of BA resulted in 3–41 female flowers per inflorescence, reaching the highest average of 23.9 at 160 mg/L BA treatment. There were 3–22 inflorescences with induced female flowers per branch on the trees treated with various concentrations of BA, and the highest average of 13.8 was observed at 20 mg/L of BA treatment. The average number of fruits per infructescence was 3.3 in the trees treated with the optimal concentration of BA (20 mg/L), compared with 1.3 for infructescences of the control trees. The results of this study show that BA is a plant growth regulator with the potential to induce floral feminization and promote fruiting of *P. volubilis*.

1. Introduction

Plukenetia volubilis, also known as Sacha inchi or Inca peanut, is a perennial oilseed vine belonging to the Euphorbiaceae family, native to the rainforests of South America (Hamaker et al., 1992). The seeds of *P. volubilis* contain 25–27% protein and 41–54% oil, which comprises approximately 90% unsaturated fatty acids (oleic, linoleic, linolenic) and is rich in vitamins E and A. The oil of *P. volubilis* has great potential economic value in cosmetic, pharmaceutical, and food industries (Guillén et al., 2003; Krivankova et al., 2007; Moser et al., 2007; Cai, 2011). Additionally, *P. volubilis* oil is a promising resource for biofuel production (Zuleta et al., 2012), and its shells can be used to synthesize a silver nanocatalyst (Kumar et al., 2014). While the composition and properties of *P. volubilis* seeds are relatively well known (Hamaker et al., 1992; Sathe et al., 2002; Gutiérrez et al., 2011; Chirinos et al., 2013), little is known about its flower development, especially sex differentiation. *P. volubilis* is monoecious with separate male and female flowers on the same inflorescence. There are about 60 small male flowers, which arrange in narrow raceme-like inflorescences, with only one or two female flowers near the base of the inflorescences. The paucity of female flowers may be one of the main factors influencing the yield potential of *P. volubilis*. Thus, developing a method to increase the number of female flowers will be helpful in improving the yield of this crop.

Plant hormones are essential factors for alternative or plastic sexual development in various plant species. Several hormones, such as cytokinin, ethylene, gibberellins, and auxins have been used successfully to convert male to female flowers in many plant species (Golenberg and West, 2013). In this work, we investigated the effects of exogenous application of 6-benzyladenine (BA, a synthetic compound with cytokinin activity) on floral feminization and fruit yield of *P. volubilis*.

2. Materials and methods

2.1. Plant materials and treatments

The experiment was carried out using 6-month-old *P. volubilis* trees, grown in the Xishuangbanna Tropical Botanical Garden

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(21°54′ N, 101°46′ E, 580 m asl), Chinese Academy of Sciences (Mengla County, Yunnan Province, China). The trees were planted with 2 m × 2 m spacing, and supported by steel wires. We selected 30 uniform trees at the beginning of the flowering stage for each treatment.

A stock solution (1 mg/ml) of BA (Bio Basic Inc., Toronto, Ontario, Canada) was prepared by dissolving 1 g BA in 8 ml 1 M NaOH and bringing the final volume to 1 L with distilled water. Tween-20 (Polysorbate-20, Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., Shanghai, China) was added to BA working solutions at a final concentration of 0.05% (v/v) as a wetting agent. Working solutions of various concentrations of BA (5, 10, 20, 40, 80, and 160 mg/L) were sprayed onto entire trees with a hand sprayer, wetting the tree to the point of run-off (approximately 300 ml BA working solution per tree). Control trees were sprayed with 300 ml distilled water containing 0.05% (v/v) Tween-20 and 1.28 mM NaOH (i.e., NaOH concentration equivalent to that in the 160 mg/L BA working solution). Spraying was conducted once at dusk. The experiments were carried out from July to September 2013.

2.2. Collection of flower and fruit data

The experiment had a completely randomized design with seven experimental treatments, with three replications of each treatment, and 10 trees per replicate. Ten similar new branches per tree were chosen to count the number of female flowers, poly-female inflorescences (>2 female flowers per inflorescence), and fruits. The numbers of female flowers per inflorescence and poly-female inflorescences per branch were counted 1 month after the BA treatments. A female flower was defined as a flower with pistils only. The number of fruits (diameter >2 cm) was counted on 3 infructescences (in the middle of the chosen branches) 2 months after the BA treatments.

2.3. Statistical analyses

Data were analyzed using the Statistical Product and Service Solution (SPSS) version 16.0 software (SPSS Inc, Chicago, IL, USA). The significance of differences among means was determined using one-way ANOVA with Tukey's post hoc tests.

3. Results and discussion

3.1. BA converted male flowers of P. volubilis into female flowers

The number of female flowers per inflorescence was significantly higher on BA-treated *P. volubilis* trees than on control trees (Fig. 1). After treatment with BA, many female flowers appeared at the position where the male flowers would normally be located (Fig. 1A and B). Some bisexual flowers with stamens and pistols formed at the upper part of the inflorescence. Each BA-treated inflorescence produced 3–41 female flowers, whereas there were only 1–2 female flowers on control inflorescences. The number of female flowers on BA-treated inflorescence increased with increasing concentrations of BA, reaching an average of 23.9 at 160 mg/L

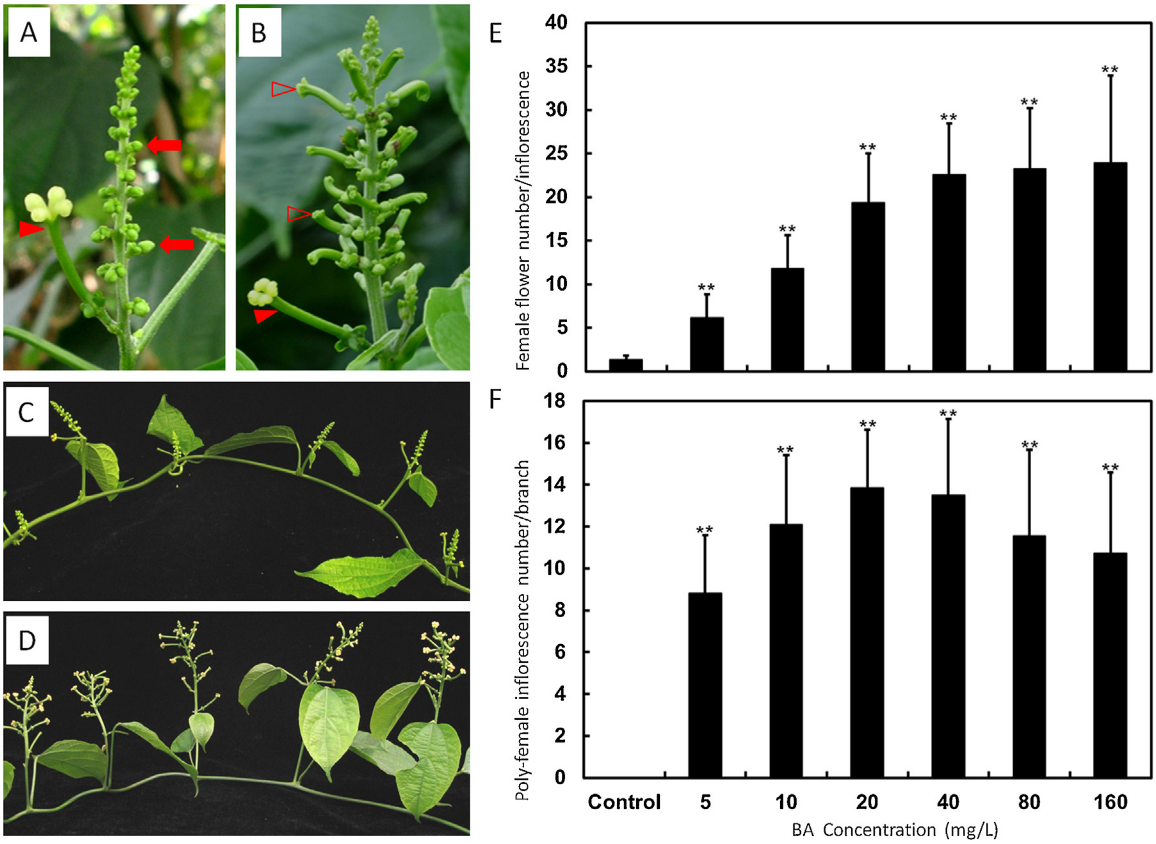

Fig. 1. BA-mediated conversion of male flowers into female flowers on *P. volubilis* inflorescences. (A) Inflorescence of control trees; (B) inflorescence of trees treated with 20 mg/L BA; (C) branch with inflorescences (control); (D) branch with poly-female inflorescences (from trees treated with 20 mg/L BA); (E) number of female flowers per inflorescence after treatment with BA at indicated concentrations; (F) number of poly-female inflorescences (>2 female flowers per inflorescence) per branch after treatment with BA at indicated concentrations. Arrows indicate male flowers; filled arrowheads indicate native female flowers; open arrowheads indicate BA-induced female flowers. Values are the mean of 30 trees ± SE, with three independent biological replicates. Significant differences between treatments and control were determined using Tukey's test. **, statistically significant at $p < 0.01$.

of BA (Fig. 1E). Treatments with high concentrations of BA (80 or 160 mg/L) resulted in the conversion of all of the male flowers to female flowers on some inflorescences. Most of the female flowers induced by the 20 or 40 mg/L BA treatments showed normal morphology. Treatments with high concentrations of BA (80 or 160 mg/L) resulted in an increase in the number of abnormal female flowers, although these treatments induced more female flowers than did the low-concentration BA treatments. The abnormal female flowers induced by high concentrations of BA had a curved style and/or a closed stigma. Some of the abnormal female flowers were wrapped by petals, and could not develop into fruits.

Numerous studies have shown that several phytohormones have masculinizing or feminizing effects in various species. Ethylene was shown to be the key feminization-determining hormone in cucumber (Ando et al., 2001; Grumet and Taft, 2011). Gibberellic acid was reported to have a masculinizing effect on *Spinacia oleracea* (Chailakhyan and Khryanin, 1978), but a feminizing effect on *Hyoscyamus niger* and maize (Resende and Viana, 1959; Dellaporta and Calderon-Urrea, 1994). Auxin was shown to have a feminizing effect on *Opuntia stenopetala* and *Cannabis sativus* (Heslop-Harrison, 1956; Orozco-Arroyo et al., 2012), but a masculinizing effect on *Mercurialis annua* (Hamdi et al., 1987).

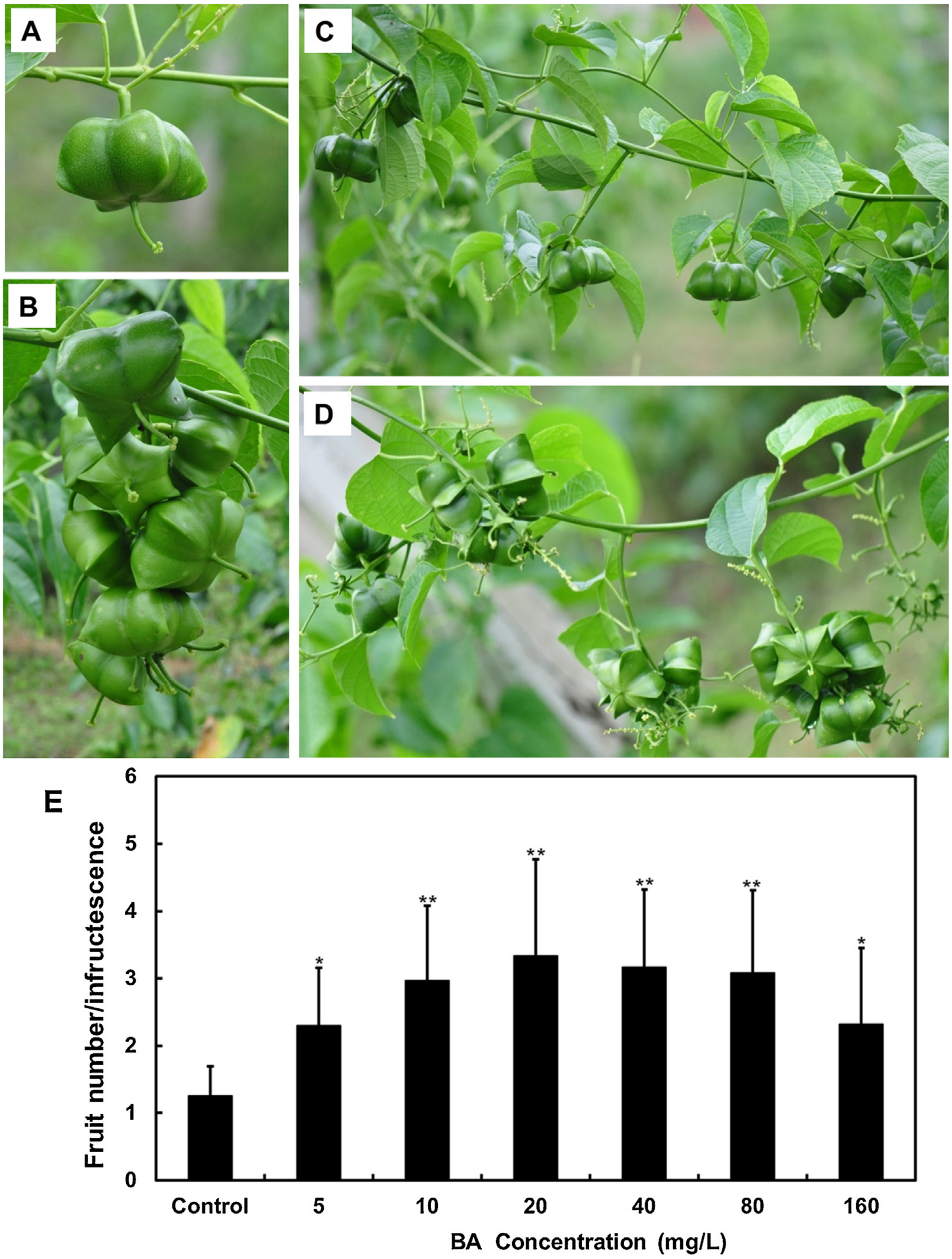

Fig. 2. Effect of BA on fruiting in *P. volubilis*. (A) Infructescence from control trees; (B) infructescence from trees treated with 20 mg/L BA; (C) fruiting branch from control trees; (D) fruiting branch from trees treated with 20 mg/L BA; (E) number of fruits (diameter >2 cm) per infructescence after treatment with indicated concentrations of BA. Values are the mean of 30 trees ± SE, with three independent biological replicates. Significant differences between treatments and control were determined using Tukey's test. ** and *, statistically significant at $p < 0.01$ and $p < 0.05$, respectively.

As reported in several studies, cytokinin has a feminizing effect on flowers of diverse species including *Mercurialis annua*, *Vitis vinifera*, *Luffa cylindrica*, and *Pinus densiflora* (Negi and Olmo, 1966; Dauphin-Guerin et al., 1980; Takahashi et al., 1980; Wakushima et al., 1996). The results of the present study are consistent with those findings. Treatment with BA was also found to induce more female flowers and bisexual flowers in *Jatropha curcas* and *Ricinus communis*, both of which are in the same family as *P. volubilis* (Euphorbiaceae) (Pan and Xu, 2011; Tan et al., 2011).

The BA treatments induced many poly-female inflorescences (>2 female flowers per inflorescence), which were not observed on control trees (Fig. 1C and D). Approximately 3–22 poly-female inflorescences per branch formed on trees treated with various concentrations of BA, with the highest average number (13.8) in the 20 mg/L BA treatment (Fig. 1F). High concentrations of BA (80 or 160 mg/L) damaged the young branches, resulting in the withering of shoot tips and fewer poly-female inflorescences on newly developing branches.

3.2. *BA treatment significantly increased fruiting of P. volubilis*

The number of female flowers is a critical determinant of fruit number in monoecious plants. Approximately 8–20% of the BA-induced female flowers further developed into fruits (diameter >2 cm), which resulted in more fruits per infructescence than the control (Fig. 2). There were only 1–2 and average of 1.3 fruits per infructescence in the control (Fig. 2A, C and E), whereas BA treatments resulted in the production of 1–7 fruits per infructescence, with an average of 3.3 fruits in the 20 mg/L BA treatment (Fig. 2B, D and E). The number of fruits increased with increasing concentrations of BA from 5 mg/L to 20 mg/L, but decreased as the concentration of BA increased from 40 mg/L to 160 mg/L (Fig. 2E). Treatment with 20 mg/L BA produced the highest number of fruits per infructescence (Fig. 2E). Although the poly-female inflorescences were continuously distributed on branches (Fig. 1D), the poly-fruit infructescences (>2 fruits per infructescence) were unevenly distributed (Fig. 2D). This was probably because of an uneven allocation of photosynthetic products and/or a poor nutrient supply.

The results of the present study indicate that cytokinin may play an important role in female flower development of *P. volubilis*. Thus, there is great potential to use this plant growth regulator to improve the fruit yield of *P. volubilis* by increasing the number of female flowers. Future research should focus on whether exogenous BA can improve the seed yield of *P. volubilis*. Also, to further elucidate the molecular mechanism of cytokinin in determining floral sex in *P. volubilis*, the endogenous cytokinin levels in male and female flowers at early developmental stages should be quantified, and genes encoding key enzymes in cytokinin metabolism in *P. volubilis* should be cloned and characterized (Werner and Schmulling, 2009). Such studies will provide information that can be used to further improve the yield of *P. volubilis* via genetic engineering.

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