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Growth and yield responses of *Plukenetia volubilis* L. plants to planting density

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SUMMARY

A field experiment was conducted in a single growing season in a tropical region of China to investigate several leaf and whole-plant traits of *Plukenetia volubilis* L. plants cultivated at high (2,500 plants ha^{-1}), medium (1,667 plants ha^{-1}), and low (1,111 plants ha^{-1}) planting densities. The results showed that leaf photosynthetic variables, measured during the wet season, were not significantly affected by planting density. The highest biomass recorded at the individual plant level occurred at the low planting density and was attributed mainly to a high leaf area and a high leaf area:biomass ratio, but not to the photosynthetic rate in leaves. Plants grown at the low density had higher specific fine-root lengths (i.e., root length per root dry mass), indicating a greater ability to capture water and nutrients to enhance growth. The mean fruit yield per plant decreased with increasing planting density. However, compared to the low (2,778.8 kg ha^{-1}) and medium (4,786.0 kg ha^{-1}) planting densities, the total fruit yield at the highest density (8,721.5 kg ha^{-1}) increased by 213.9% and 82.2%, respectively, throughout the growing season. The increase in total fruit yield was largely due to an increase in the number of fruit per unit area, and not to increased fruit size, which compensated for the accompanying decrease in the productivity of each plant. Meanwhile, the protein content and fatty acid profile of seed kernels collected at the peak period of fruit ripening were not significantly affected by planting density. However, the oil content of seed kernels increased significantly with an increase in planting density, presumably by increasing the source:sink ratio. These results indicate that increasing the number of fruit per unit area might be an effective means to increase seed yield and oil production in *P. volubilis* plants in order to meet an increasing demand for this oil.

Oilseeds provide a major source of dietary calories for humans and are an increasingly significant source of renewable industrial material. Globally, the major oilseed crops (i.e., rapeseed, soybean, sunflower, maize, palm, linseed, and olive) contribute > 90% of all vegetable oils used for food, feed, and industrial applications. However, as the global population is growing rapidly, the requirement for food and edible oils is increasing (Gui *et al.*, 2008). The increasing demand for high quality vegetable oils has focussed research on increasing yields from other oilseed crops (Dyer *et al.*, 2008; Bharucha and Pretty, 2010).

Plukenetia volubilis L. is native to South America and is a promising new oilseed crop in the family Euphorbiaceae. *P. volubilis* is a perennial woody vine that produces seeds with high protein (27 – 30%) and oil (40 – 60%) contents, exceeding the quality characteristics of existing oils used for worldwide human consumption (Cai, 2011a; Fanali *et al.*, 2011). The use of *P. volubilis* seed as a source of oil for domestic, industrial, medicinal, and cosmetic uses has increased the economic importance of this promising crop worldwide (Cai, 2011a). *P. volubilis* plants do not experience Winter dormancy and grow continuously in tropical regions. Therefore, they flower and fruit almost continuously throughout the year. The fruits are capsules (*ca.* 4 – 7 cm in diameter) consisting of four-to-seven pods, with one seed per pod.

Although the biochemical composition and properties of *P. volubilis* seeds are relatively well-known, it is important to increase fruit and seed yields, and oil contents, for commercial-scale oil production. The processes determining the quantity and quality of seed oil in *P. volubilis* plants are variable and depend on cultivar, environmental conditions, and agronomic factors (Semino *et al.*, 2008; Cai, 2011b; Jiao *et al.*, 2012). Planting densities aimed at maximising yields per unit area affect the requirements of plants for above-ground (light) and below-ground (water and nutrient) resources, and thus affect the growth and yield of the crop (Cox, 1996; Brahim *et al.*, 1998; Andrade *et al.*, 2005). However, the effects of planting density on biomass, fruit yield, and seed size vary from species to species. In many species, increased planting density, up to a certain optimum level, resulted in increased yields (Brahim *et al.*, 1998). In some species, a lower planting density resulted in a prolonged seeding-fill period and thus increasing seed size (Rogers and Lomman, 1988).

As a relatively new oilseed crop, *P. volubilis* plants have been cultivated empirically at intra- and inter-row spacings of 2.0 m and 3.0 m, respectively (Semino *et al.*, 2008). To date, no optimum planting density has been established for maximum fruit or seed production. In the

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present work, a field experiment was conducted to investigate the effects of planting density on the growth characteristics, fruit yields, and seed quality of *P. volubilis* plants during a single growing season in Xishuangbanna, a tropical region of China. The overall goal was to provide a better understanding of the management needs of this species at both the local and regional levels.

MATERIALS AND METHODS

Experimental site, plant material, and experimental treatments

Seeds of *P. volubilis* were sown in a nursery in February 2011. When the seedlings were approx. 15 cm tall, uniformly-sized seedlings were selected and over 10,000 plants were cultivated at an open site in mid-April 2011 at Puwen (22°03' N, 101°76' E; 880 m asl), Xishuangbanna, Southeast Yunnan, P. R. China, with three intra- and inter-row spacings: 2.0 m and 2.0 m; 2.0 m and 3.0 m (traditional); or 3.0 m and 3.0 m, respectively. The climatic characteristics of the experimental site were 1,260 mm total annual precipitation, with considerably higher amounts of rainfall in the wet season (June to October) and an annual average air temperature of 22.7°C. The average air temperature in February 2012 was 18.9°C (lowest 7.5°C) and was 27.8°C (highest 38.5°C) in May 2011 (Figure 1). Soil samples were collected in October 2011. The characteristics of the top layer of soil (0 – 20 cm) were: pH 5.48; organic carbon content 5.95% (w/v); total nitrogen content 0.35 g kg^{-1}; available N content 47 mg kg^{-1}; available P content 14.9 mg kg^{-1}; and available K content 27 mg kg^{-1}.

The experiments were conducted between September 2011 and April 2012. Plots were arranged in a randomised complete block design consisting of the three planting density treatments, each with three plots. Twenty-five to 28 plants were examined per plot. The traditional planting density of *P. volubilis* plants has a 2 m inter-row spacing, with 3 m between plants, resulting in a density of 1,667 plants ha^{-1}. The other planting densities tested here were 2,500 or 1,111 plants ha^{-1}. Only the central plants in each plot were considered for evaluation, to avoid edge effects. Since *P. volubilis* is a liana species, all plants were supported to a height of 1.6 m using steel wire. Fertilisation consisted of 100 kg ha^{-1} of a 1:1:1 (w/w/w) mix of N:P:K spread in a *ca.* 2 m-wide zone in June 2011.

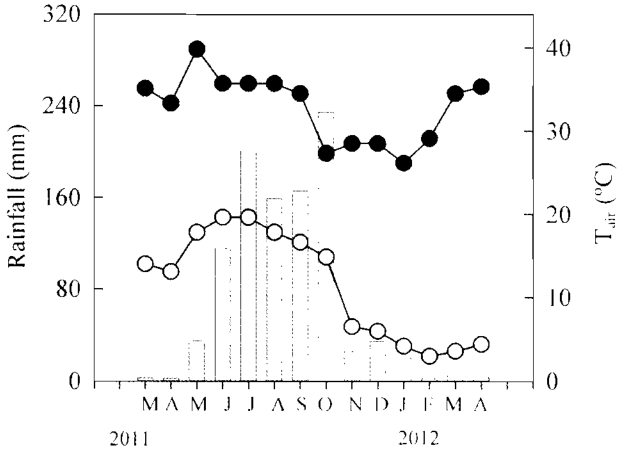

FIG. 1

Monthly precipitation (grey bars) and maximum (closed circles) and minimum (open circles) air temperatures during the 14-month growing period studied (2011-2012).

Measurements of leaf and whole-plant traits

At the end of September 2011 (the middle of the wet season), photosynthetic parameters were measured on fully-expanded, recently-matured, sun-canopy leaves using a portable infrared gas analyser operating in the open system mode (LI-6400XT; Li-Cor, Lincoln, NE, USA) between 08.00 – 11.30 h at an ambient CO_2 concentration of 380 µmol mol^{-1}. Leaf temperature and the vapour pressure deficit (VPD) in the cuvette were kept at 25° – 26°C and < 1.0 kPa, respectively. Four-to-five plants were selected in each of the three replicate plots for photosynthetic measurements at each planting density. One leaf was measured per plant.

Photosynthetic light response curves were developed based on measurements at nine photosynthetic photon flux density (PPFD) values ranging from 0 – 2,000 µmol m^{-2} s^{-1} using an LED-B lamp (Li-Cor) as the internal actinic light source. Photosynthetic rate *vs.* PPFD response curves were fitted to a non-rectangular hyperbola. From these curves, the asymptotic light-saturated rate of net photosynthesis (A_{max}), apparent quantum yield (AQY), dark respiration rate (R_d), near-light saturation point, and light compensation point were determined. Stomatal conductance (g_s) was measured on the same leaves under light-saturating irradiance. Intrinsic water-use efficiency (WUE_i) was calculated as the ratio of A_{max} to g_s.

Four-to-six plants were harvested from each treatment in each replicate plot in late October 2011. The stem diameter at 2 cm above soil level was measured using callipers. The plants were separated into leaves, stems, coarse roots (diameter ≥ 1.0 mm), fine roots (diameter < 1.0 mm), flowers (both male and female parts), and fruit (almost all green). All were dried to constant mass and weighed. The number of fruit per plant was also counted.

Sub-samples of leaves and fine roots were scanned on a desktop scanner (CanoScan 4400F; Canon, Melville, NY, USA). The images were analysed for leaf area, fine-root length, and average fine-root diameter using DT-SCAN image analysis software (Delta-T Devices, Cambridge, UK). The leaf area of each sample was measured and converted to a leaf area index (LAI). Specific leaf area values (SLA; leaf area in cm^2 g^{-1} leaf), the leaf area ratio (LAR; leaf area in cm^2 g^{-1} plant), and the source:sink ratio (leaf area in cm^2:fruit number) were calculated.

Mature fruit from *P. volubilis* plants were harvested manually from all three planting densities six-times in each replicate plot throughout the 4-month fruit ripening period. At each harvest, total fruit numbers per plot were counted, and fruit dry mass (DM; "size") was measured. Total fruit yields, the numbers of fruit per unit area (ha^{-1}), and mean fruit DM ("size") were compared between the three different planting densities.

Determination of protein, total lipid, and fatty acid compositions

Samples of seeds (30 – 40 g) were collected in February 2012, the peak fruit ripening period. The seeds were broken open to remove the shells and the seed kernels were sent to the Kunming Center for Inspection and Testing for Quality and Safety of Agricultural Products, Ministry of Agriculture, P. R. China, to analyse their lipid, protein, and fatty acid compositions. The total nitrogen content of each seed kernel sample was determined using the standard micro-Kjeldahl method and was converted to a protein content using a conversion factor of 6.25. Total lipid contents and fatty acid profiles were measured using an automated gas chromatograph (Model 6890; Hewlett Packard Co., Palo Alto, CA, USA) equipped with a flame ionisation detector, as described previously (Cai *et al.*, 2012).

Statistical analysis

All data are presented as mean values ± standard deviations (SD). Analyses were performed using the SPSS statistical software package (Version 16.0; SPSS Inc., Chicago, IL, USA) and the significance ($P \leq 0.05$) of the data was analysed by one-way ANOVA (Tukey's test).

RESULTS AND DISCUSSION

Leaf and individual whole-plant traits

The net light-saturated photosynthetic rate (A_{max}) of *P. volubilis* leaves was relatively high (19.8 – 21.6 µmol m^{-2} s^{-1}) during the wet season. Planting density had no significant effect on any of the leaf photosynthetic variables measured, including A_{max}, stomatal conductance, dark respiration, and the intrinsic water-use efficiency (ANOVA; all $P > 0.05$; Table I). In contrast, Cox (1996) found that as the planting density increased, the rate of leaf CO_2 exchange declined in modern maize (*Zea mays*) hybrids. Narrow row-spacing usually increases light interception because it provides a better spatial distribution of plants (Andrade *et al.*, 2005). However, the appropriate planting arrangement depends on the growth characteristics of the species (and/or genotype) such as plant height, growth habit, and plant architecture (Andrade *et al.*, 2005). As a liana species, *P. volubilis* plants were artificially supported by steel wires to a fixed height. Planting density therefore did not change the plant architecture, and thus did not lead to variations in canopy leaf-level carbon gain throughout plant development in the growing season.

Planting density had a significant effect ($P \leq 0.05$) on all whole-plant morphological traits, except fine-root diameter (Table II). *P. volubilis* plants increased their basal stem diameter and total biomass at the low planting density (Table II), which has been reported previously (Leach *et al.*, 1999; Soratto *et al.*, 2012). This result was due mainly to the greater availability of growing space and reduced competition between plants within the rows, thus allowing plants to accumulate higher amounts of photo-assimilates for growth. Growth parameters such as leaf area, specific leaf area (SLA), and the leaf area ratio (LAR), in particular, could be related to plant biomass production (dry weight; DW) and development. The decrease in SLA at a lower planting density indicated that the leaves became thicker as part of an adaptive mechanism to the surrounding environment. Planting density significantly influenced the LAR and the leaf area index (LAI), probably by improving infrastructural traits and the photosynthetic efficiency of leaves (Andrade *et al.*, 2005; Cai, 2011b). Compared to the low planting density, reduced leaf area and LAR values, combined with a similar level of photosynthesis, resulted in lower photosynthetic yields and, consequently, lower biomass (DW) production per plant at the high planting density.

Biomass production may also depend on root system traits, since plants that grow rapidly are usually associated with higher specific fine-root length values (SRL; i.e., root length per root DM) and smaller root diameters which enable plants to acquire more below-ground resources (Cai, 2011b). Although data on root growth and below-ground competition for available space are lacking at the different planting densities, high SRL values for *P. volubilis* plants grown at the low planting density indicated a competitive advantage in terms of the rapid acquisition of water and nutrient resources. Overall, the patterns of leaf and fine-root traits observed in the present study contributed to an effective strategy for maximising the capture of above- and below-ground resources (i.e., light, water, and nutrients) for individual *P. volubilis* plants grown at a low planting density. Consequently, these plants showed higher potential growth rates than plants grown at the high planting density.

The numbers of fruit per unit area and the DM of both fruits and flowers (male and female combined) per plant decreased, whereas the estimated source:sink ratio increased at the high planting density (Table II). The higher DM of fruit per plant was accompanied by higher leaf area and plant biomass values. If carbohydrate biosynthesis is considered to be a consequence of the translocation of photo-assimilates, our results suggested that fruit development in *P. volubilis* plants grown at a high planting density was source-limited. Plants grown at a high density had a lower photosynthetic input, due mainly to having smaller leaf areas and less stored carbohydrate from the maternal plant during the reproductive stage. Thus, fewer resources were available for reproductive (i.e., flower and seed) production (Cai *et al.* 2012).

TABLE I

Photosynthetic parameters in P. volubilis *plants cultivated at three different planting densities in the wet season*

Planting density[‡]	A_{max}[§] (µmol m^{-2} s^{-1})	g_s[§] (mol m^{-2} s^{-1})	AQE[§] (mol CO_2 m^{-1} photon)	R_d[§] (µmol m^{-2} s^{-1})	LCP[§] (µmol m^{-2} s^{-1})	LSP[§] (µmol m^{-2} s^{-1})	WUE$_i$[§] (µmol mol^{-1})
HD	19.8 ± 1.7[†]	0.582 ± 0.104	0.043 ± 0.004	2.12 ± 0.39	48.7 ± 5.54	509.0 ± 45.7	34.0 ± 2.9
MD	19.8 ± 0.87	0.599 ± 0.044	0.0469 ± 0.003	2.64 ± 0.61	56.0 ± 9.45	478.8 ± 20.3	33.0 ± 1.4
LD	21.6 ± 0.79	0.701 ± 0.083	0.0482 ± 0.003	2.87 ± 0.93	59.2 ± 18.26	508.3 ± 21.3	30.8 ± 1.1

[†] Values are means (n = 4 or 5) ± SD. No significant differences were noted in each column at $P > 0.05$ by Tukey's test.

[‡] HD, high planting density (2,500 plants ha–1); MD, medium planting density (1,667 plants ha^{-1}); LD, low planting density (1,111 plants ha^{-1}).

[§] A_{max}, light-saturated photosynthetic rate; g_s, stomatal conductance; WUE$_i$, intrinsic water use efficiency; AQE, apparent quantum efficiency; R_d, dark respiration rate; LCP, light compensation point; LSP, light saturation point.

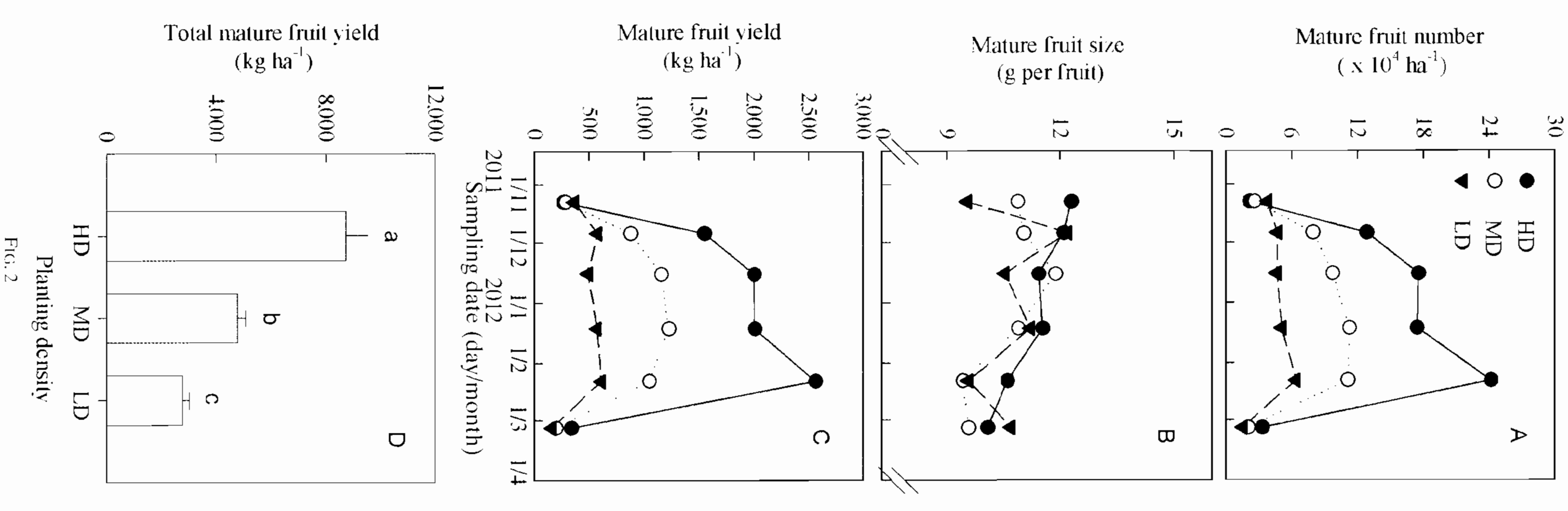

FIG. 2

Mature fruit number (Panel A), fruit size (Panel B), fruit yield (Panel C), and total mature fruit yield (Panel D) during the growing season in *P. volubilis* plants cultivated at three different planting densities. Mean values (n = 3) with a different lower-case letter (Panel D) indicate significant differences between planting densities at $P \leq 0.05$. HD, high density (2,500 plants ha^{-1}); MD, medium density (1,667 plants ha^{-1}); LD, low density (1,111 plants ha^{-1}).

TABLE II

Whole-plant traits in P. volubilis *plants cultivated at three different planting densities*

Planting density[‡]	Total biomass (kg)	Stem diameter (cm)	Leaf area (m^2)	LAI[§]	SLA[§] (cm^2 g^{-1})	LAR[§] (cm^2 g^{-1})	SRL[§] (m g^{-1})	RD[§] (mm)	Fruit number (no. per plant)	Source:sink ratio (leaf area/fruit number)	Flower biomass (g)	Fruit biomass (kg)
HD	4.10 ± 0.74b[†]	1.68 ± 0.27b	16.3 ± 8.4b	4.1 ± 1.1a	236.7 ± 6.7b	29.9 ± 4.0b	12.6 ± 3.6b	0.70 ± 0.09a	213.8 ± 49.2c	7.7 ± 1.9a	27.8 ± 12.5b	1.48 ± 0.61b
MD	4.91 ± 0.07b	1.81 ± 0.30b	19.5 ± 3.2a	3.3 ± 0.53ab	224.5 ± 12.6b	38.1 ± 7.5a	12.7 ± 3.5b	0.64 ± 0.08a	380.5 ± 36.8b	5.3 ± 0.61b	47.8 ± 9.4a	1.88 ± 0.35b
LD	6.64 ± 0.37a	2.03 ± 0.24a	20.0 ± 4.5a	2.2 ± 0.50b	210.1 ± 8.6a	39.7 ± 5.6a	21.3 ± 12.4a	0.70 ± 0.09a	471.5 ± 53.4a	4.4 ± 0.64b	48.0 ± 12.4a	3.09 ± 0.55a

[†] Mean values (n = 4 - 6) ± SD in each column followed by a different lower-case letter indicate significant difference at $P \leq 0.05$ by Tukey's test.

[‡] HD, high planting density (2,500 plants ha^{-1}); MD, medium planting density (1,667 plants ha^{-1}); LD, low planting density (1,111 plants ha^{-1}).

[§] SLA, specific leaf area; LAR, leaf area ratio; LAI, leaf area index; SRL, specific fine-root length; RD, fine-root diameter.

TABLE III

Protein content, oil content, and fatty acid profile of the seed kernels of P. volubilis *plants cultivated at three different planting densities*

Planting density[‡]	Lipid content (%)	Protein content (%)	Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Eicosenoic acid (%)	SFA[§] (%)	UFA[§] (%)
HD	51.6 ± 1.07a[†]	30.6 ± 0.95a	4.44 ± 0.22a	3.51 ± 0.37a	10.38 ± 1.17a	36.49 ± 1.91a	44.19 ± 2.66a	0.42 ± 0.04a	7.95 ± 0.54a	91.48 ± 0.53a
MD	47.8 ± 1.20b	32.6 ± 1.28a	3.97 ± 0.24a	3.16 ± 0.27a	10.22 ± 0.49a	35.42 ± 1.05a	46.21 ± 1.24a	0.46 ± 0.04a	7.13 ± 0.27ab	92.31 ± 0.29a
LD	47.6 ± 2.13b	32.4 ± 1.50a	4.08 ± 0.38a	2.89 ± 0.17a	9.62 ± 0.31a	35.18 ± 0.67a	46.96 ± 0.36a	0.44 ± 0.05a	6.97 ± 0.55b	92.20 ± 0.60a

[†] Values are means (n = 4) ± SD. Values in each column followed by different lower-case letters indicate significant difference at $P \leq 0.05$ by Tukey's test.

[‡] HD, high planting density (2,500 plants ha^{-1}); MD, medium planting density (1,667 plants ha^{-1}); LD, low planting density (1,111 plants ha^{-1}).

[§] SFA, saturated fatty acids; UFA, unsaturated fatty acids. All values are % (w/w).

Fruit yield per plot and seed quality

Patterns in the numbers and yields of mature fruit at the whole plot level (i.e., ha^{-1}) were similar between the three planting densities, with relatively high values observed from December to February (Figure 2A, C). It is well-documented that fruit (or pod) number is a critical factor for seed yield in oilseed crops (Andrade *et al.*, 1999; Peltonen-Sainio and Jauhiainen, 2008). Hasanuzzaman (2008) found that there was a significant positive relationship between pod number and seed yield in rapeseed (*Brassica campestris*) at both the individual plant and plot levels. On the other hand, Ozer (2003) suggested that seed size (i.e., the 1,000 seed weight) was another primary factor that determined the seed yield of winter oilseed rape (*Brassica napus*).

The present study indicated that fruit size (i.e., DM per fruit) in *P. volubilis* plants did not differ between the three planting densities across the different sampling dates (Figure 1B). Thus, the increased number of fruit ha^{-1} with increasing planting density was mainly responsible for the influence of planting density on total fruit yield throughout the growing season. This was consistent with the data from long-term (1976 – 2006) studies on oilseed rape cultivation analysed by Peltonen-Sainio and Jauhiainen (2008). A positive relationship was also found between the number of fruit and fruit yield ha^{-1} across all sampling dates (r = 0.99; $P \leq 0.001$). Compared to the low (2,778.8 kg ha^{-1}) and medium (4,786.0 kg ha^{-1}) planting densities, the mean total fruit yield of *P. volubilis* plants at the high planting density (8,721.5 kg ha^{-1}) was significantly increased, by 213.9% and 82.2%, respectively (Figure 2D). Thus, increasing the number of fruit produced ha^{-1} may be one of the most effective ways to increase fruit yields and oil production in *P. volubilis* plants. As the planting density increased, increases in the numbers of fruit and yields at the plot level were able to compensate for the accompanying decrease in productivity in individual plants. In contrast, in a series of multi-factorial field experiments, Leach *et al.* (1999) found that seed yields of winter oilseed rape increased with planting density up to 50 – 60 plants m^{-2}. The relationship between planting density and grain yield is usually depicted as a curve, rising to a maximum, then showing a slow decline at higher planting densities (Momoh and Zhou, 2001; Zhang *et al.*, 2012). The minimum planting density giving the highest biological yield may also be the density giving the maximum grain yield. Therefore, we predict that there is potential to increase fruit and seed yields in *P. volubilis* plants by increasing the planting density, within a certain range.

Among the different planting densities, oil (47.6 – 51.6%) and protein (30.6 – 32.6%) contents, and unsaturated fatty acid (UFA; 91.48 – 92.31%; oleic, linoleic, eicosenoic and linolenic combined) concentrations in seed kernels were comparable to those in previous reports (Fanali *et al.*, 2011; Cai *et al.*, 2012; Jiao *et al.*, 2012). Seed kernel oil contents generally increased with increasing planting density, which was consistent with results from winter oilseed rape (Zhang *et al.* 2012) and *Lesquerella fendleri* (Brahim *et al.*, 1998), but differed from the results of Momoh and Zhou (2001) who found that the seed oil content of winter oilseed rape decreased significantly with increasing planting density. Meanwhile, there have also been reports that the seed oil content of castor bean (*Ricinus communis*; Soratto *et al.*, 2012), sunflower (*Helianthus annuus*; Narwal and Malik, 1985), and Winter oilseed rape (Leach *et al.*, 1999) were not affected by planting density. According to these authors, the influence of environmental factors and growth traits on the seed oil contents of oilseed crops was poorly understood, as the results diverged from one experiment to another, which may have been due to differences in varieties, and/or planting densities, as well as interactions between these factors. The oil contents of the seeds of some crops exhibit high and constant heritability (Soratto *et al.*, 2012), whereas in others oil contents are quite variable (Cai *et al.*, 2012; Zhang *et al.*, 2012). The higher kernel oil content of *P. volubilis* plants at the high planting density might be due to the fact that the estimated source:sink ratio increased significantly with planting density (Trentacoste *et al.*, 2010). As the oils are mainly based on carbon compounds, an increased supply of photo-assimilates may enhance the synthesis of fatty acids and thus increase the oil content of seeds (Voelker and Kinney, 2001).

In conclusion, the higher accumulations of biomass (FW) at the individual plant level observed in *P. volubilis* plants grown at a low planting density (1,111 plants ha^{-1}) was mainly attributable to higher leaf area and LAR values, not to a higher photosynthetic rate. As the planting density increased, the increase in total fruit yield at the plot level, due to increased numbers of fruit, was able to compensate for the decrease in productivity of individual plants. Meanwhile, seed kernel oil contents increased significantly with increasing planting density. A relatively high planting density of *P. volubilis* plants is required to optimise seed oil yields in this new oilseed crop.

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