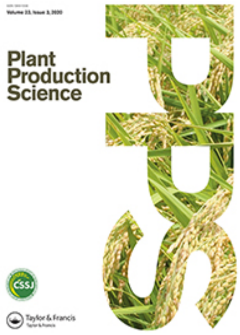

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Enhancing root lodging resistance of maize with twin plants in wide-narrow rows: a case study

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ABSTRACT
Root lodging is known to reduce the yield and quality of maize, which will be more serious driven by the changes in agriculture such as the higher planting density and the more extreme precipitation events. Here we describe a new cultivation method to reduce the root lodging of maize. We designed two planting layouts: twin plants (TP) and single plant (SP) in a hole with the same density. The vertical root-pulling resistance, angle and rate of natural root lodging, root and shoot morphology related to root lodging and maize yield were compared between two planting layouts. TP planting significantly increased the vertical root-pulling resistance and angle of natural root lodging. This can be partly attributed to the gripping force between the staggered crown roots of the two adjacent plants. Moreover, the TP planting could increase root-lodging resistance by increasing the root angle (acute angle between the stem direction and root) and stem diameter. Additionally, TP planting did not reduce the maize yield and biomass. Consequently, our study demonstrated that the twin plants in a hole are effective to decrease the root lodging of maize in southwest of China. This technique is simple, inexpensive, safe, stable, and has broader potential for increasing maize yield and quality. Twin plants layout in wide-narrow rows significantly increased the vertical root-pulling resistance of maize, which mainly attributed to the gripping force between the staggered crown roots of the two adjacent plants.

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Twin plants layout

1 Introduction

Maize (*Zea mays* L.) is one of the most important crops for providing food, industrial raw materials and feed. However, it is known that maize production is extremely sensitive to root lodging (Bian et al., 2016; Chen, Yang & Chu, 1990; Liu, Song, Liu, Zhu & Xu, 2012; Stamp & Kiel, 1992). Root lodging often causes considerable yield losses of maize. For example, the yield of three maize hybrids decreased by 25-39% due to a storm with a maximum wind speed of 19.2 m/s (So, Adetimirin & Kim, 2013). Yield reduction is potentially most severe when root lodging occurs around flowering time (Berry et al., 2004; Minami & Ujihara, 1991). Moreover, root lodging also decreases grain and straw quality of maize, inducing low mineral and protein contents (Berry et al., 2004) and harmful toxins such as aflatoxin (Langseth & Stabbetorp, 1996), causing a great challenge for food safety.

Importantly, the root-lodging risk of maize is increasing globally. First, the extreme precipitation and storm events are increasing (Altieri, Nicholls, Henao & Lana,

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Supplemental data for this article can be accessed here.

2015; Wasko & Sharma, 2015). Second, the soil degradation, caused by soil erosion, heavy machinery rolling and intensive land use, is increasing. The degraded farmland can reduce the root growth and anchorage of maize (Berry et al., 2004). Third, the more and more simple cultivation techniques, such as no inter-tillage and no hill up cultivation (especially in China), are increasing the risk of root lodging. Besides, increasing N fertilisation in agricultural systems may cause serious root lodging for maize (Chen et al., 1990; Crook & Ennos, 1995). The last but not the least, increasing planting density as an agronomic practise is being adopted to meet the high yield demand in many regions of the world (Xue et al., 2017). Current plant density in most regions is ≥7 plants m^{-2}. However, high density can cause the lodging of maize roots (Liu et al., 2012). Consequently, it is imperative to explore how the root lodging can be reduced to a sustainability production for maize.

To reduce the yield and quality decreasing of cereal caused by root lodging, many techniques have been developed, which can be divided into two categories. One category is to dwarf the plant height or improve the morphology related to root anchorage (such as nodal root number and growth angle). This category method is operated by genetic breeding (Bruce, Folkerts, Crasta & Folkerts, 2001) or the application of growth regulators (Zhang, Yu, Zhang, Zhou & Tan, 2017; Zhang et al., 2014), which has attracted much attention since the late 1960s (Berry et al., 2004), and is well known as green revolution. Another category is cultivation management, which can co-ordinate most environmental factors, including light (Hébert et al., 2001), soil moisture and nutrients (Chen et al., 1990) and soil physical characteristics (Bian et al., 2016) to decrease root lodging. To our knowledge, the effective cultivation management for reducing maize root lodging mainly includes the decreased crop density (Liu et al., 2012), tillage increasing the anchorage strength of roots (Bian et al., 2016; Chen et al., 1990), nutrient management with increasing applications of potassium (K) or decreasing N fertilizers (Chen et al., 1990; Haegele, Becker, Henninger & Below, 2014), suitable water and wind management, the control of diseases and insect pests which damage roots (Sutter, Fisher, Elliott & Branson, 1990) and altering sowing dates and depth (Dai et al., 2017; Pinthus, 1967). However, the effective and adopted widely cultivation management techniques are still scarce. A simple, effective and adaptive technique for decreasing maize root lodging is necessary.

Plant arrangement within fields is an important cultivation technique. The arrangement for maize population could include the rows and plants within the row. Traditionally, the space between rows is constant. This design can increase light penetration for maize plants grown under low or medium density. However, when planting density is greater, the design forms a closed canopy, leading to severe competition between plants, low radiation use efficiency (Liu, Song & Zhu, 2012a) and poor permeability within the canopy. These can hinder exchanges of water vapour and heat (Liu & Song, 2012b) and lead to increase plant disease and insect problems (Ballaré, 2014). As an alternative strategy, the wide-narrow row arrangement was designed and adopted in many regions of the world. But investigations reported that this configuration cannot increase maize yield at very high planting densities, such as ≤~80,000 plants ha^{-1} (Haegele et al., 2014; Robles, Ciampitti & Vyn, 2012) or even 75,000 plants ha^{-1} (Xue, Ma & Lu, 2002). This may be partly due to overcrowding between plants within a row (Jiang et al., 2013). The number of plants per hole is a major technique for the arrangement of plants within a row. Increasing the number of plants in each hole, and increasing hole spacing may relieve the crowd stress at a constant density. Studies have shown that the arrangement of two plants per hole can increase maize yield (Nafziger, 1996), especially under wide-narrow row conditions (Wei et al., 2011; Xue et al., 2002). These works suggested that the twin plants in wide-narrow rows layout may be an important way to increase planting density without decreased crop yields, or to increase yields in high-density systems. However, whether this design will lead to serious root lodging is uncertain and merits investigation is necessary.

Given that root lodging is the permanent displacement or breakage of the root system in the soil, leading plants to collapse or crawl along the ground (Ennos, 1991), many studies focused on relationships between root morphology and maize lodging. These studies showed that root-lodging resistance is positively correlated with higher numbers of roots (Stamp & Kiel, 1992), greater root diameter, enlarged nodal root layer and shallower growth angles of nodal roots (Pinthus, 1967). However, most studies focused on the plant level and separated roots from adjacent plants (Bian et al., 2016; Brune, Baumgartenand, Mckay, Technow & Podhiny, 2017; Liu et al., 2012). Thus, previous studies did not explore relationships between root growth and lodging resistance beyond the plant level. At the plant level, the architecture of root systems is configured by axial and lateral roots, forming an overall streamlined spindle structure. But at the population level, root systems may act as an interconnected web, woven by the roots of different plants. If the mechanical characteristics of the root structure between two levels exist, then it may be possible to design new systems decreasing root lodging based on the web root structure. For maize, the twin plants in a wide-narrow rows layout close to each other, we can postulate whether the root growth behaviour of the two adjacent plants will avoid each other or interlace, and how root morphology and architecture will change. In turn, we might ask whether these processes affect the vertical root-pulling resistance of maize, which is strongly correlated with root-lodging resistance (Kamara, Kling, Menkir & Ibikunle, 2003).

The objectives of the present study were to determine root-lodging resistance of maize under the planting layout of single plant and twin adjacent plants per hole in wide-narrow rows layout (Figure 1). We focused on root vertical root-pulling resistance, natural root-lodging survey, root and shoot morphology related to root lodging and crop yield. Our hypotheses were that: (i) roots grow in a staggering pattern in relation to each other in the twin plants layout, which increases root vertical root-pulling resistance and this decreases the risk of natural root lodging; (ii) the twin plants layout improves root and shoot morphology related to root lodging; and (iii) maize productivity (biomass and yield) are increased or not reduced by the twin plants layout.

2 Materials and methods

2.1 Experimental site

Field experiments were conducted during April–October in both 2015 and 2016, at the Daheqiao Agricultural Experimental Station, Yunnan Agricultural University, Xundian, Yunnan Province, south-west China (103°16′41″ E, 25°31′07′N). This site is located at an altitude of 1860 m, with a mean annual temperature of 14.7°C, mean annual sunshine duration of 2119 h, and mean frost-free period of 229 days. Mean annual precipitation is 1045.0 mm in the last three years and mainly falls during May–September. The field soil is a silty clay loam paddy soil (Chinese Classification). The soil (0–20 cm depth) had a pH of 7.64 with total N, phosphorus (P) and K contents of 0.08, 17.49 and 2.16 g kg^{-1}, respectively. Available N, P and K were 53.07, 0.82 and 117.25 mg kg^{-1}, respectively. The soil organic matter content was 17.9 g kg^{-1}. The former crop was broad beans.

2.2 Experimental design

The local maize variety (Yunrui 99, a lodging susceptible cultivar) was chosen for field experiments. This cultivar is widely used in south-west China. We designed two planting layouts: single plant (SP) and twin plants (TP) per hole in wide-narrow rows layout (Figure 1). In SP, the spacing was 23 cm, while in TP the spacing was 46 cm and the distance between two adjacent seeds in a hole was 0–3 cm. The rows layout was designed with 80 cm wide row plus a 40 cm narrow row. The rows were oriented in a north–south direction. The two planting layouts had the same density (72,464 plants ha^{-1}), which is a high density for maize production in China (Zhang et al., 2014) and is susceptible to root lodging (Bian et al., 2016). Each experiment plot (treatment) was 6 m long (14 rows with two as guard ones) by 4.5 m wide (the plants at the end of each row were used as guard plants), and replicated three times. The plots were randomly arranged in three blocks on the experimental land (20 m length x 10 m width). Maize seeds were over-sown by hand on 24/05/2015 and 17/05/2016, and thinned at the third-leaf stage. During the experiment, crops were protected against weeds, insects and diseases, as required, and were irrigated artificially to prevent water stress. The controlled release fertilizer (790 kg ha^{-1}, N:P:K = 24:6:10) was mixed evenly into the soil by rotary tillage prior to sowing, but no additional fertilizer was added after that treatment.

2.3 Plant samples and measurements

2.3.1 Vertical root-pulling resistance

The vertical root-pulling resistance was measured on 20/08/2015 and 15/08/2016. At this period, the maize was at

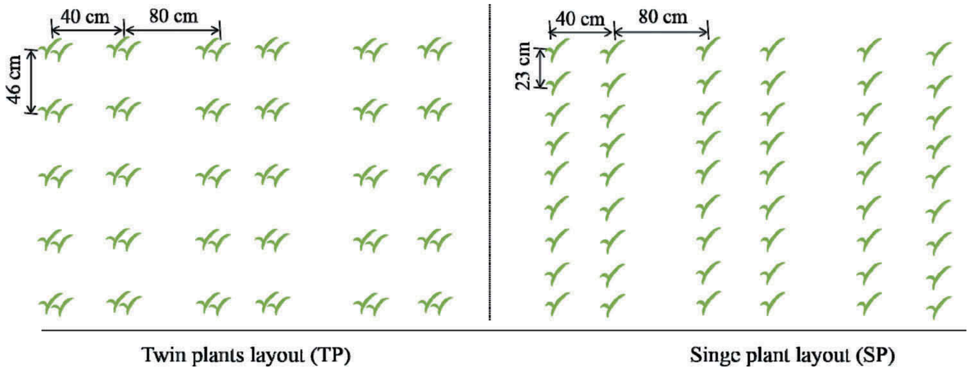

Figure 1. The maize planting layout of the twin plants (TP) and single plant layout in wide-narrow rows (SP).

the milk stage, which is a susceptible time for root lodging of cereals and associated loss of crop yield (Kamara et al., 2003). To do this, we pre-wetted the soil of half of each plot (7 rows x 4.5 m row length, with 1 guard row) 48 h before measurements and ensured that the soil was fully wetted to 30 cm depth. Non-neighboring plants were randomly selected and artificially lodged using the 3yc-1 vertical root-pulling instrument (produced by Jilin Academy of Agricultural Sciences, Institute of Maize in China; maximum range 2450 N). A similar instrument was used in other studies and proved reliable in measuring plant root-lodging resistance (e.g. Bian et al., 2016). When we measured the vertical root-pulling resistance, the leaves and sheaths were removed, then the stem was cut off at 20–25 cm above the ground. We operated the equipment to record the maximum force of vertical root-pulling after the whole root system was extracted from the ground. A detailed description of the system is provided by Bian et al. (2016). Additionally, to measure possible synergistic resistance exerted by the two adjacent plants in the TP treatment (twin plants in each hole), we separately measured the vertical root-pulling force of the single plant alone (TPS) or both of the two adjacent plants at the same time (TPD). Therefore, the number of sample plants in each TP treatment was 30 and 15 plants per plot in SP. Broken plants were discarded from the analysis. In 2016, after measuring vertical root-pulling force, we measured the surface area of the mixed mass of soil extracted from the ground. To do this, the area was regarded as a quadrangle and the length of both the parallel and vertical directions of rows was recorded to determine the area.

2.3.2 Natural root lodging

On 11-12/07/2016 (maize growth at eight-leaf stage), a storm (maximum wind speed 25 m s^{-1}, maximum precipitation intensity 60 mm h^{-1}) caused natural root lodging. This provided an opportunity to investigate natural root lodging (on 13/07/2016), although the yield reduction caused by root lodging could be slight at this time (Berry et al., 2004). We measured the angle and rate of root lodging. For the angle, a plastic plate (1 m x 0.8 m) and a large protractor were used. We then recorded the angle when one side of the protractor was parallel and close to the ground, while the other side was close to the plant stem. All plants in each block were designated as lodged when the stem angle (from the original upright orientation of the plant) was more than a 45° angle (Novacek, Mason, Galusha & Yaseen, 2013), and the angles were recorded. We then evaluated the lodging rate for each plot.

2.3.3 Plant morphology related to lodging and crop productivity

After measuring vertical root-pulling resistance, stems were cut off at ground level, then the natural plant height and first ear height were measured. Stem diameter (at the centre of the second stem segment) was measured using a vernier caliper. Leaves, stems and cobs were separately oven-dried at 70°C to constant weight to determine dry weight (15 plants each plot). Moreover, the whole root system of plants (8 plants per plot in 2015 and 10 in 2016) was pulled out, excess soil was removed in the field and then the roots were carefully washed with tap water to remove soil adhering to roots. Then root morphology was measured. The nodal roots of maize, especially the above-ground brace roots, are especially important for supporting shoots (Liu et al., 2012; Stamp & Kiel, 1992). We first measured the horizontal spread length of root systems in four directions (two parallel directions of the row and two vertical directions of the row) and recorded the maximum and mean values. Then, we measured the growth angle, number and diameter of nodal roots for each whorl separately. The first and second whorl nodal roots at the bottom of the stem were defined as the first (P1), second phytomer (P2) in turn, and so on (Sylvain, 1993). Only nodal roots that penetrated into the soil were considered when root morphology was measured. In this study, we mainly measured the nodal roots above the P1 layer, which may be particularly important for root anchorage. The growth angle of nodal roots was measured with a protractor made with thin plastic (2 mm thickness), which can be easily inserted into the narrow crack between nodal roots. When the protractor was inserted vertically into the crack between roots, the acute angle between the stem direction and root (at 5 cm from the root base) was measured. In each layer of nodal roots, 5 roots were measured (all roots if < 5 roots) at symmetrical directions, apart from the bare section. After growth angles were measured, each layer of nodal roots was separately cut using scissors. The number of roots was counted and root diameters were measured using a vernier caliper positioned 5 cm from the root base. Finally, all roots (including the primary root) were oven-dried at 60°C for 48 h, to determine dry weight.

At the crop maturity stage (10/10/2015 and 05/10/2016), the maize in the remaining half of the plot (7 rows x 4.5 m row length, with 2 guard rows) was harvested by hand to obtain fresh weight. Ten ears in each plot were randomly selected and divided into kernel and cob. Fresh weight was measured and then samples were oven-dried at 60°C to constant weight. Then, the ratio of kernels to ears and kernel to moisture was determined. The final grain yield was adjusted to 14.0% moisture content.

2.4 *Data analysis*

Data were analysed by Analysis of Variance (ANOVA) using SPSS statistical software (version 21). Fixed factors were: (1) planting layout (for lodging rate and yield), (2) both planting layout and block (for lodging angle and all root morphological characteristics related to lodging and crop growth) and (3) both planting layout or vertical root-pulling style (pulling on the single plant in SP treatment, the single plant alone in TP treatment and both of the two adjacent plants at the same time in TP treatment) and block (for the vertical root-pulling resistance of roots). The normality and the homogeneity of variable variances were previously verified, and, if necessary, natural logarithms (log) were taken of raw data to meet homoscedastic and normality requirements. A posteriori LSD test was used to identify significant ($P < 0.05$) differences among treatments.

3 Results

3.1. *Vertical root-pulling resistance*

Compared to the layout of a single plant in each hole (SP), the arrangement of twin plants layout (TP) significantly increased the vertical root-pulling resistance of maize in both 2015 ($F = 18.801$, $P < 0.01$) and 2016 ($F = 22.658$, $P < 0.01$) (Figure 2). In the TP treatment, when we pulled the roots of one single plant alone (TPS), the other plant was also simultaneously extracted (Figure 3(c,d)). Moreover, the difference of vertical root-pulling resistance between TPS and TPD was not significant in both 2015 ($F = 0.264$, $P = 0.612$) and 2016 ($F = 1.478$, $P = 0.233$). This proved the observation that there was a gripping force between the twin plants, because the roots of both plants were enmeshed (Figure 3(c)). Further evidence of the greater vertical root-pulling resistance in TP was the differences in

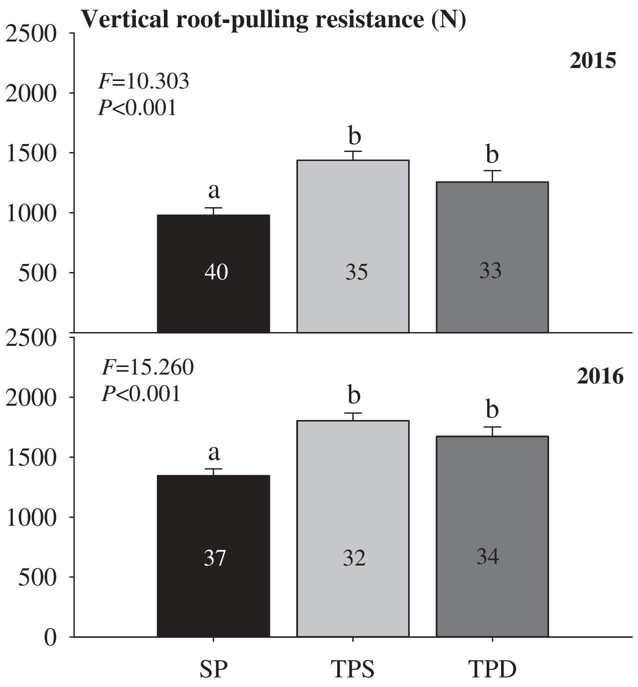

Figure 2. Effect of planting layout on vertical root-pulling resistance of maize in both 2015 and 2016 (means ± SE). SP = vertical root-pulling of single plants in planting pattern of single plant; TPS = vertical root-pulling of single plants in TP; TPD = vertical root-pulling of twin plants at the same time in TP. The different lower-case letters among treatments are significantly different at $P < 0.05$ (Duncan's Multiple Range Test). The number within each bar denotes the number of measurements (n).

Figure 3. (a) Maize roots of two adjacent plants in the TP system. (b) and (c) Both the two plants in TP were extracted from the ground at the same time. (d) Comparing the mass of soil body between SP and TP when extracted from the ground.

the horizontal area of the mixed body of soil and extracted from the ground, which in TP (950 ± SE 37 cm^2) was significantly higher than in SP (780 ± SE 41 cm^2) ($F = 9.212$, $P < 0.01$; Figure 3(b)). Similarly, the mean root spread length in TP (8.09 ± SE 0.22 cm) was not significantly higher than in SP (7.79 ± SE 0.30 cm) ($F = 0.622$, $P = 0.434$).

3.2. Natural root lodging

On 11-12/07/2016, a storm occurred when the maize had grown to the eight-leaf stage, which caused root lodging. Then, natural root lodging was investigated, in terms of rate and angle. We found that there was no significant difference on root-lodging rate between SP (28%) and TP (9%) ($F = 0.239$, $P = 0.650$, Figure 4(a)). However, the root-lodging angle (acute angle between the stem direction and ground) was significantly higher in the TP system (49.93°) than in the SP system (45.56°) ($F = 9.155$, $P = 0.003$, Figure 4(b)), which partly confirmed that twin plants layout increase vertical root-pulling resistance.

3.3. Root morphology related to lodging

In both 2015 and 2016, the planting layout had significant effects on root morphology related to lodging (Table 1). In 2015, the growth angle in the vertical direction of the fourth nodal root in the SP arrangement was significantly lower than that in TP system ($F = 6.820$, $P < 0.001$), but no significant difference was found in the other nodal layers. In 2016, the root growth angle in SP was significantly lower than that of in TP at the second ($F = 46.450$, $P < 0.001$), third ($F = 33.097$, $P < 0.001$) and fourth ($F = 19.856$, $P < 0.001$) nodal layers. However, in both 2015 and 2016, we did not find significant differences between TP and SP in terms of root number (except the fifth nodal root in 2016), root diameter and root biomass. There was no significant difference on maximum root spread length between TP (10.16 ± 0.52 cm) and SP (9.74 ± 0.53 cm) ($F = 0.307$, $P = 0.582$).

3.4. Shoot morphology related to root lodging and crop productivity

The plant morphology and crop productivity were significantly affected by TP (Table 2). Plant height ($F = 7.170$, $P = 0.010$) and stem diameter ($F = 18.576$, $P < 0.001$) were significantly higher in TP than SP in 2015, but not in 2016. Compared to SP, TP significantly increased the yield of maize in both 2015 (15%, $F = 6.486$, $P = 0.014$) and 2016 (13%, $F = 4.521$, $P = 0.039$). TP also significantly increased the biomass of maize in both 2015 (12%, $F = 6.094$, $P = 0.017$) and 2016 (13%, $F = 6.014$, $P = 0.018$). There was no significant difference between TP and SP on ear height. In both 2015 and 2016, the TP obviously leads to poor uniformity with higher C.V. on ear weight and biomass but not on plant height and stem diameter (Table 3).

4. Discussion

Our two-year field study showed that changing planting layout from a single plant (SP) to twin plants (TP) per hole can substantially increase root-lodging resistance of maize in artificial test and partly in field investigations. The increased root vertical root-pulling resistance in TP mainly attributed to the gripping force between two adjacent maize plants. Moreover, the TP did not reduce maize yield as it was reported by previous studies (Nafziger, 1996; Wei et al., 2011; Xue et al., 2002). Therefore, the finding suggested that TP planting layout can be used as an effective technology to enhancing the root-lodging resistance of maize, and may be helpful to facilitate the application of double plants method which not be used widely in maize production.

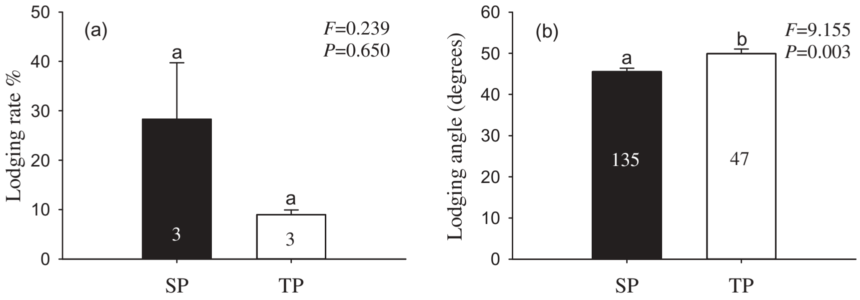

Figure 4. Effect of planting layouts on (a) natural lodging rate and (b) natural lodging angle of maize at eight-leaf stage in 2016 (means±SE). Different lower-case letters between treatments are significantly different at $P < 0.05$. Lodging occurred with rainfall (maximum precipitation intensity 60 mm h^{-1}) combined with a wind (maximum wind speed 25 m s^{-1}). The number within each bar denotes the number of measurements (n).

Table 1. Effects of planting layout on root morphology related to lodging in 2015 and 2016. Values are means ± SE. SP = planting pattern of single plant in each hole; TP = planting pattern of twin plants in each hole. Nodal roots were measured from the second to fifth whorl, from bottom towards the top. Bold text denotes a significant difference in root morphology between SP and TP ($P < 0.05$). The 'n' in table denotes the number of measurements.

Year	Root parameters	Nodal root layer	SP	*n*	TP	*n*	*F*	*P*
2015	Number (plant^{-1})	Second	8.25 ± 1.25	23	8.2 ± 0.86	23	0.001	0.974
		Third	7.25 ± 1.25	22	7.00 ± 0.84	21	0.030	0.868
		Fourth	7.75 ± 0.94	20	7.20 ± 0.97	20	0.159	0.702
		Fifth	8.50 ± 1.50	23	7.00 ± 0.63	19	1.000	0.351
	Diameter (mm)	Second	2.16 ± 0.10	24	1.91 ± 0.08	22	3.431	0.068
		Third	3.21 ± 0.11	21	2.94 ± 0.08	21	3.678	0.060
		Fourth	3.92 ± 0.11	24	3.85 ± 0.07	23	0.365	0.548
		Fifth	4.65 ± 0.11	20	4.39 ± 0.10	19	2.435	0.124
	Angle (°)	Second	36.2 ± 1.26	23	31.04 ± 1.74	22	2.473	0.087
		Third	38.3 ± 1.03	23	37.14 ± 1.61	22	1.755	0.177
		Fourth	39.3 ± 1.19	22	41.02 ± 1.58	21	6.820	**<0.001**
		Fifth	47.5 ± 2.28	14	48.00 ± 1.71	15	1.724	0.231
	Biomass (g plant^{-1})	Second	1.83 ± 0.40	22	1.59 ± 0.30	23	0.233	0.644
		Third	3.69 ± 0.76	21	2.71 ± 0.38	20	1.537	0.255
		Fourth	9.80 ± 1.46	22	7.34 ± 1.10	20	1.930	0.207
		Fifth	11.22 ± 1.74	19	8.90 ± 0.50	17	1.724	0.231
2016	Number (plant^{-1})	Second	5.81 ± 0.31	26	6.36 ± 0.44	25	1.071	0.306
		Third	7.25 ± 0.33	28	7.04 ± 0.39	26	0.172	0.680
		Fourth	11.25 ± 0.42	28	11.62 ± 0.47	26	0.342	0.561
		Fifth	13.36 ± 0.67	28	15.19 ± 0.58	26	4.110	**0.048**
	Diameter (mm)	Second	2.07 ± 0.13	27	2.12 ± 0.13	25	0.085	0.772
		Third	3.37 ± 0.11	28	3.24 ± 0.12	25	0.624	0.433
		Fourth	4.30 ± 0.10	28	4.43 ± 0.12	25	0.783	0.380
		Fifth	4.83 ± 0.14	28	5.01 ± 0.17	24	0.725	0.400
	Angle (°)	Second	31.52 ± 1.62	27	47.98 ± 1.79	25	46.450	**<0.001**
		Third	30.56 ± 1.49	27	43.27 ± 1.63	26	33.097	**<0.001**
		Fourth	36.50 ± 1.46	27	46.10 ± 1.57	26	19.856	**<0.001**
		Fifth	44.50 ± 2.10	22	49.70 ± 2.40	17	2.656	0.133
	Biomass (g plant^{-1})	Second	1.88 ± 0.27	28	2.69 ± 0.25	17	3.282	0.078
		Third	7.63 ± 0.63	29	9.33 ± 0.86	16	1.227	0.275
		Fourth	11.62 ± 1.70	28	12.72 ± 2.68	16	1.279	0.265
		Fifth	18.60 ± 3.77	18	26.88 ± 5.16	12	3.051	0.098

Table 2. Effects of planting layout on growth traits and yield of maize in 2015 and 2016. Values are means ± SE. SP = planting pattern of single plant in each hole; TP = planting pattern of twin plants in each hole. Bold text denotes significant differences in growth traits related to root lodging and yield between SP and TP ($P < 0.05$). The 'n' in table denotes the number of measurements.

Year	Growth parameters	SP	*n*	TP	*n*	*F*	*P*
2015	Yield (kg ha^{-1})	8435.5 ± 273.3	3	9685.5 ± 383.6	3	6.486	**0.014**
	Biomass (g plant^{-1})	258.23 ± 8.02	40	290.61 ± 9.88	43	6.094	**0.017**
	Plant height (cm)	263.09 ± 3.16	40	273.43 ± 2.36	43	7.170	**0.010**
	Stem diameter (mm)	24.23 ± 0.46	40	26.78 ± 0.39	43	18.576	**<0.001**
	Ear height (cm)	152.87 ± 3.29	40	145.82 ± 2.32	43	3.228	0.079
2016	Yield (kg ha^{-1})	8833.2 ± 204.8	3	10,057.6 ± 197.4	3	4.521	**0.039**
	Biomass (g plant^{-1})	226.96 ± 9.22	42	314.12 ± 11.90	44	6.014	**0.018**
	Plant height (cm)	240.71 ± 1.79	42	244.04 ± 2.21	44	1.367	0.248
	Stem diameter (mm)	22.45 ± 0.33	42	22.69 ± 0.41	44	0.218	0.642
	Ear height (cm)	110.72 ± 1.76	42	108.64 ± 2.16	44	0.555	0.460

Table 3. The coefficient of variation (C.V.) on growth traits in 2015 and 2016. SP = planting pattern of single plant in each hole; TP = planting pattern of twin plants in each hole. The data for each treatment are calculated from the sample plants in all plots.

Year	Treatments	Plant height	Ear weight	Biomass	Stem diameter
2015	SP	0.057	0.155	0.150	0.089
	TP	0.046	0.210	0.180	0.077
2016	SP	0.090	0.228	0.166	0.090
	TP	0.085	0.282	0.243	0.085

The TP planting technology appears effective in decreasing root lodging and whether it should be practicably adopted in maize production. Currently, technology for decreasing maize root and stem lodging mainly includes dwarfing plants using genetic technology or chemical control techniques (Zhang et al., 2014), variety selection (Bruce et al., 2001), cultivation of lower crop densities (Liu et al., 2012), nutrient management, such as decreasing N fertilizer applications (Chen et al., 1990), disease and pest control (Sutter et al., 1990), and using suitable tillage to promote root anchorage strength (Bian et al., 2016). However, these technologies may have some defects. For example, the application of genetic technology may be restricted due to environmental complexity, diversity and variability; some chemical control techniques, if dosage is excessive, may be unsafe and damage the environment; decreasing N fertilizer use cannot be achieved in the short term, due to increasing food demands (Godfray et al., 2010); decreasing crop density may not be realized, because high maize yields greatly depend on high-density planting (Testa, Reyneri & Blandino, 2016); increasing potash fertilizer use is expensive and may lead to soil nutrient imbalances. However, these defects may not exist in TP. Therefore, TP could be simple, inexpensive, safe and suitable. Moreover, it can also be used with the existing technologies. Besides, the TP can further increase or do not reduce maize yields, which may trigger broader adoption. Nevertheless, broader adoption needs investigations with more varieties and crop types.

Two force processes can explain why the layout of twin plants can decrease root lodging of maize. Firstly, when two adjacent maize plants grow together, their nodal roots can stagger and form a root net in the soil, which can induce a gripping force to promote root anchorage (Figures 2 and 3). Moreover, the staggered root growth between the twin maize plants may physically enlarge the root plate by connecting the roots of both plants, which promotes synergistic strengthening of root anchoring (Manzur, Hall & Chimenti, 2014; Piñera-Chavez, Berry, Foulkes, Jesson & Reynolds, 2016). Therefore, the TP can be used on crops with large crown root systems, but not be suitable on some cereals (e.g. wheat) or tap root crops (e.g. beans) without whorl nodal roots, especially the air roots in the staggered condition. Consequently, the suitability of TP planting technology in other crops requires study. In fact, even maize in twin plants layout cannot substantially increase vertical root-pulling resistance if the whorl nodal root did not develop. This can partly explain why the difference of natural root-lodging rate between SP and TP was not significant because the maize just growth at eight-leaf stage when the nodal root of maize is not developed. Nevertheless, the natural lodging angle at eight maize leaf was significantly decreased by twin plants layout, which can indicate that twin plants layout could improve root-lodging resistance., Secondly, under high and consistent density conditions, the TP system increases the space between adjacent holes within a row, which may promote canopy ventilation and provide space for the flexible stem to recover from crushing, thus decreasing the transmission and accumulation of wind energy between plants in the canopy (Schindler, 2008). Consequently, wind energy can be better absorbed in windy conditions. This process may explain regular root lodging in high plant density cereal crops (Liu et al., 2012). Nevertheless, we should concern that TP increase the aboveground biomass and wind area on the plants in a hill. This process contrarily increase the risk of root lodging by wind. In fact, the wind loads on two plants in a hill depend on the balance of the two processes, which closely related to the space between hills. Therefore, studies are needed to determine how much space between hills in TP are suitable for producing a greater anchorage force at belowground but smaller wind loads at aboveground and its delivery to roots (namely, greater root-lodging resistance).

Beyond the direct effects on the force causing roots lodging, the plant morphology is related to maize lodging. Firstly, many studies showed that the root morphology can significantly affect maize root lodging (Pinthus, 1967; Stamp & Kiel, 1992). However, we did not find significant differences between TP and SP in terms of root number (except the fifth nodal root in 2016), root diameter, root biomass, maximum root spread length between and mean root spread length. Although TP significantly increased the root growth angle in 2016, which could increase root anchorage (Pinthus, 1967), but this significant effect did not emerge in 2015 (except fourth nodal root), which may driven by environmental growth conditions (Bian et al., 2016). Consequently, the increased resistance to root lodging in our study cannot be mainly attributed to the differences in root morphology, but to the interlacing nodal roots between two adjacent maize plants. The gripping force from the interlacing nodal roots stably increases root-lodging resistance in 2 years. Secondly, because wind forces were transferred from shoots to roots, stem and canopy properties have important effects on root lodging. For example, thin stems and high plant or ear height can make plants more susceptible to root lodging (Bian et al., 2016; Kamara et al., 2003). In 2015 but not 2016, our data showed that TP planting layout can significantly increase stem diameter (Table 2), which may increase root-lodging resistance. However, we found TP increased plant heights, but the greater plant heights could not increase root lodging, as biomass and stem diameter also increased (Table 2). Therefore, increased plant height did not lead to thinner stems, as can occur when there is competition for light. Moreover, we found no significant difference on stem diameter, plant height and ear height between two planting layouts. Thirdly, soil physical properties affect root lodging of crop, especially soil compaction (Berry et al., 2004) and soil moisture content (Ibrahim et al., 1993). During a storm, the interaction between wind and soil water is the key driver for root lodging (Kamimura, Kitagawa, Saito & Mizunaga, 2012). TP can change the spatial arrangement of plants and canopy configuration. This process may change the soil water content, runoff and soil erosion, and precipitation redistribution (Liu et al., 2015; Mohammed & Gumbs, 1982), altering the hydrological properties and soil strength of the rhizosphere. Consequently, if TP changes the mechanical characteristics of the root–soil interface merits further investigation in a broader range of agro-climatic conditions.

There were two interesting questions could merit to be concerned. For the first question, TP had significantly higher yields and biomass compared to SP, which suggests that using TP did not lead to a intense competition between the adjacent plants. Then, why the competition did not increase when the distance between the adherent plants in TP closed to 0–3 cm. The reason might be due to the increased light transmission, stomatal conductance, intercellular CO_2 concentration, transpiration rate and net photosynthetic rate in TP with the greater gaps between two planting holes and the border effect (Wang et al., 2015; Wei et al., 2011). Although the competition between twin plants can lead to poor uniformity but the strong competitor will offset some of the yield lose from the weak one through compensatory growth. Moreover, the leaves between the two adjacent plants could utilize the gap spaces, driven by shading at the seeding stage (Maddonni, 2002). This process may be restricted in SP, as adult plant cannot easily turn their leaves direction horizontally, but can only adjust the upward growth angle of leaves. So, the TP can be especially important to increase planting density without decreased crop yields, or to increase yields in high-density systems. Similarly, roots, especially the lower layer nodal root and primary root between the two adjacent TP plants, unilaterally forage the soil space around them (Cahill et al., 2010), which will enlarge the root distribution range in the soil. In turn, this could compensate for the intense competition in the overlapping area. Plants can increase the absorption of soil nutrients when the nutrients distribution is patchy (Loecke & Philip, 2009). Thus, nutrient use in TP may be greatly increased, as the concentration of nutrients in the patch will be doubled when it was applied in the hole under the same fertilizer rate. However, some previous studies reported that the variability of plant spacing reduced the maize yield. These studies created the variability of plant spacing by thinning or surveying, and without considering the effect of density (Chim et al., 2014; Pommel & Bonhomme, 1998). Therefore, the reduced yield may not be caused by the twin plant (doubles), but by the different plant density. In fact, the gaps and doubles (main factors causing the variability of plant spacing) within a row were opposite to affect the maize yield. Gaps reduced yield while doubles increased yield (Nafziger, 1996). Moreover, the most previous studies used the equivalent row spacing, but not the wide-narrow rows in our study. In the wide-narrow row with same density, non-uniformity with doubles can benefit the light transmission in canopy, and did not reduce the maize yield (Xue et al., 2002). For the second question, the roots of the twin plants grow and overlap within the same soil space. More maize roots may increase plant exudates and accelerate decomposition processes. This can foster various harmful flora and fauna, in turn damage the anchorage function of roots (Sutter et al., 1990). However, we did not find any obvious root damage in either SP or TP in both 2015 and 2016 (Figure 3(a)). This may be a interesting question need to be explore for TP in more environments.

5. Conclusions

In our experiment site, the study demonstrated that the planting layout of twin plants in wide-narrow rows layout (TP) can significantly increase the vertical root-pulling resistance of maize and angle of natural root lodging. This partly to be a function of the gripping force between the crown roots of the two adjacent plants. Moreover, the TP planting increased the root angle (acute angle between the stem direction and root) and stem diameter, which related to the root-lodging resistance. Additionally, TP did not reduce maize yield. Therefore, our study provided an effective and acceptable planting technique to control root lodging for maize in southwest of China. Moreover, compared to the other existing techniques for controlling root lodging, TP planting layout is simple, lowcost, safe, stable. Thus, this technique will be valuable in a changing agriculture increasing the risk of root lodging on maize, and may be helpful to facilitate the application of double plants method.

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References

Altieri, M. A., Nicholls, C. I., Henao, A., & Lana, M. A. (2015). Agroecology and the design of climate change-resilient farming systems. *Agronomy for Sustainable Development*, *35*, 869–890.

Ballaré, C. L. (2014). Light regulation of plant defense. *Annual Review of Plant Biology*, *65*, 335–363.

Berry, P. M., Sterling, M., Spink, J. H., Baker, C. J., Sylvester-Bradley, R., Mooney, S. J., … Ennos, A. R. (2004). Understanding and reducing lodging in cereals. *Advances in Agronomy*, *84*, 217–271.

Bian, D., Jia, G., Cai, L., Ma, Z., Eneji, A. E., & Cui, Y. (2016). Effects of tillage practices on root characteristics and root lodging resistance of maize. *Field Crop Research*, *185*, 89–96.

Bruce, W., Desbons, P., Crasta, O., & Folkerts, O. (2001). Gene expression profiling of two related maize inbred lines with contrasting root-lodging traits. *Journal of Experimental Botany*, *52*, 459–468.

Brune, P. F., Baumgartenand, A., Mckay, S. J., Technow, F., & Podhiny, J. J. (2017). A biomechanical model for maize root lodging. *Plant and Soil*, *11*, 1–12.

Cahill, J. F., McNickle, G. G., Haag, J. J., Lamb, E. G., Nyanumba, S. M., & Clair, C. C. (2010). Plants integrate information about nutrients and neighbors. *Science*, *328*, 1657.

Chen, S. S., Yang, T. C., & Chu, T. M. (1990). Reduction of maize lodging by irrigation, tillage and nitrogen application. *Journal of Agriculture & Forestry*, *39*, 167–174.

Chim, B. K., Omara, P., Macnack, N., Mullock, J., Dhital, S., & Raun, W. (2014). Effect of seed distribution and population on maize (*Zea mays* L.) grain yield. *International Journal of Agronomy*, *2014*, 1–8.

Crook, M. J., & Ennos, A. R. (1995). The effect of N and growth regulators on stem and root characteristics associated with lodging in two cultivars of winter wheat. *Journal of Experimental Botany*, *46*, 931–936.

Dai, X., Wang, W. C., Dong, X. C., Qian, T. F., Yin, L. J., Dong, S. X., … He, M. R. (2017). Delayed sowing can increase lodging resistance while maintaining grain yield and nitrogen use efficiency in winter wheat. *The Crop Journal*, *5*, 541–552.

Ennos, A. R. (1991). The mechanics of anchorage in wheat Triticum aestivum L.: II. Anchorage of mature wheat against lodging. *Journal of Experimental Botany*, *42*, 1607–1613.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., … Toulmin, C. (2010). Food security: The challenge of feeding 9 billion people. *Science*, *327*, 812–818.

Haegele, J. W., Becker, R. J., Henninger, A. S., & Below, F. E. (2014). Row arrangement, phosphorus fertility, and hybrid contributions to managing increased plant density of maize. *Agronomy Journal*, *106*, 1838–1846.

Hébert, Y., Guingo, E., & Loudet, O. (2001). The response of root/shoot partitioning and root morphology to light reduction in maize genotypes. *Crop Science*, *41*, 363–371.

Ibrahim, M, Erskine, W, Hanti, G, & Fares, A. (1993). Lodging in lentil as affected by plant population, soil moisture and genotype. *Experimental Agriculture*, *29*, 201–206.

Jiang, W., Wang, K., Wu, Q., Dong, S., Liu, P., & Zhang, J. (2013). Effects of narrow plant spacing on root distribution and physiological nitrogen use efficiency in summer maize. *The Crop Journal*, *1*, 77–83.

Kamara, A. Y., Kling, J. G., Menkir, A., & Ibikunle, O. (2003). Association of vertical root-pulling resistance with root lodging and grain yield in selected S1 maize lines derived from a tropical low-nitrogen population. *Journal of Agronomy and Crop Science*, *189*, 129–135.

Kamimura, K., Kitagawa, K., Saito, S., & Mizunaga, H. (2012). Root anchorage of hinoki (*Chamaecyparis obtuse* (Sieb. Et Zucc.) Endl.) under the combined loading of wind and rapidly supplied water on soil: Analyses based on tree-pulling experiments. *Europe Journal Forest Research*, *131*, 219–227.

Langseth, W., & Stabbetorp, H. (1996). The effect of lodging and time of harvest on deoxynivalenol contamination in barley and oats. *Journal of Phytopathol*, *144*, 241–245.

Liu, H., Zhang, L., Zhang, R., Zhang, L., Wang, Y., & Li, G. (2015). Stemflow of water on maize and its influencing factors. *Agricultural Water Management*, *158*, 35–41.

Liu, S., Song, F., Liu, F., Zhu, X., & Xu, H. (2012). Effect of planting density on root lodging resistance and its relationship to nodal root growth characteristics in maize (*Zea mays* L.). *Journal of Agriculture Science*, *12*, 182–189.

Liu, T., & Song, F. (2012b). Maize photosynthesis and microclimate within the canopies at grain-filling stage in response to narrow-wide row planting patterns. *Photosynthetica*, *50*, 215–222.

Liu, T., Song, S., & Zhu, X. (2012a). Light interception and radiation use efficiency response to narrow-wide row planting patterns in maize. *Australian Journal of Crop Science*, *6*, 506–513.

Loecke, T. D., & Philip, R. G. (2009). Soil resource heterogeneity in the form of aggregated litter alters maize productivity. *Plant and Soil*, *325*, 231–241.

Maddonni, G. A. (2002). Maize leaves turn away from neighbors. *Plant Physiology*, *130*, 1181–1189.

Manzur, M. E., Hall, A. J., & Chimenti, C. A. (2014). Root lodging tolerance in *Helianthus annuus* (L.): Associations with morphological and mechanical attributes of roots. *Plant and Soil*, *381*, 71–83.

Minami, M., & Ujihara, A. (1991). Effects of lodging on dry matter production, grain yield and nutritional composition at different growth stages in maize (*Zea mays* L.). *Japanese Journal of Crop Science*, *60*, 107–115.

Mohammed, A., & Gumbs, F. A. (1982). The effect of plant spacing on water runoff, soil erosion and yield of maize (*Zea mays* L.) on a steep slope of an ultisol in Trinidad. *Journal of Agricultural Engineering Research*, *27*, 481–488.

Nafziger, E. D. (1996). Effects of missing and two-plant hills on corn grain yield. *Journal of Production Agriculture*, *9*, 238–240.

Novacek, M. J., Mason, S. C., Galusha, T. D., & Yaseen, M. (2013). Twin rows minimally impact irrigated maize yield, morphology, and lodging. *Agronomy Journal*, *105*, 268–276.

Piñera-Chavez, F. J., Berry, P. M., Foulkes, M. J., Jesson, M. A., & Reynolds, M. P. (2016). Avoiding lodging in irrigated spring wheat. I. Stem and root structural requirements. *Field Crops Research*, *196*, 325–336.

Pinthus, M. J. (1967). Spread of the root system as indicator for evaluating lodging resistance of wheat. *Crop Science*, *7*, 107–110.

Pommel, B., & Bonhomme, R. (1998). Variations in the vegetative and reproductive systems in individual plants of an heterogeneous maize crop. *European Journal of Agronomy*, *8*, 39–49.

Robles, M., Ciampitti, I. A., & Vyn, T. J. (2012). Responses of maize hybrids to twin-row spatial arrangement at multiple plant densities. *Agronomy Journal*, *104*, 1747–1756.

Schindler, D. (2008). Responses of Scots pine trees to dynamic wind loading. *Agricultural and Forest Meteorology*, *148*, 1733–1742.

So, Y. S., Adetimirin, V. O., & Kim, S. K. (2013). Observational study on the recovery from root lodging at flowering time and yield reduction in maize (*Zea mays* L.). *Plant Breeding & Biotechnology*, *1*, 171–177.

Stamp, P., & Kiel, C. (1992). Root morphology of maize and its relationship to root lodging. *Journal Agronomy Crop Science*, *168*, 113–118.

Sutter, G. R., Fisher, J. R., Elliott, N. C., & Branson, T. F. (1990). Effect of insecticide treatments on root lodging and yields of maize in controlled infestations of western corn root worms (Coleoptera: Chrysomelidae). *Journal of Economic Entomology*, *83*, 2414–2420.

Sylvain, P. (1993). Rate of differentiation and emergence of nodal maize roots. *Plant and Soil*, *148*, 155–161.

Testa, G., Reyneri, A., & Blandino, M. (2016). Maize grain yield enhancement through high plant density cultivation with different inter-row and intra-row spacings. *European Journal of Agronomy*, *72*, 28–37.

Wang, Y., Zhang, Y., Song, W., Li, J., Deng, W., & Wang, Z. (2015). Grain filling and yield of summer maize under planting pattern of more plants per hole. *Journal of Maize Sciences*, *23*, 117–123. (In Chinese).

Wasko, C., & Sharma, A. (2015). Steeper temporal distribution of rain intensity at higher temperatures within Australian storms. *Nature Geoscience*, *8*, 527–529.

Wei, L., Xiong, Y., Ma, C., Zhang, H., Shao, Y., Li, P., … Wang, T. (2011). Photosynthetic characterization and yield of summer corn (*Zea mays* L.) during grain filling stage under different planting pattern and population densities. *Acta Ecologica Sinica*, *31*, 2524–2531.

Xue, J., Ma, G., & Lu, H. (2002). Studied of compact and big ear type maize Shandan 902 on population characters of sink-source and yield formation. *Acta Botanica Boreali-occidentalia Sinica*, *22*, 1336–1342. (In Chinese).

Xue, J., Xie, R., Zhang, W., Wang, K., Hou, P., Ming, B., … Li, S. (2017). Research progress on reduced lodging of high-yield and -density maize. *Journal of Integrative Agriculture*, *12*, 2717–2725.

Zhang, Q., Zhang, L., Evers, J., Werf, W. V. D., Zhang, W., & Duan, L. (2014). Maize yield and quality in response to plant density and application of a novel plant growth regulator. *Field Crop Research*, *164*, 82–89.

Zhang, W., Yu, C., Zhang, K., Zhou, Y., & Tan, W. (2017). Plant growth regulator and its interactions with environment and genotype affect maize optimal plant density and yield. *European Journal of Agronomy*, *91*, 34–43.