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*Full Length Article*

# Effect of Plant Density and Harvest Stage on Yield and Quality of *Rheum tanguticum* Maxim. ex Balf

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## Abstract

*Rheum tanguticum* is a widely used and spread medical plant on the Qinghai-Tibet plateau. However, over exploitation of *R. tanguticum* has made the storage of it sharply decreased. While, only few studies concentrated on cultivation of this medical plant. The primary purpose of this study is to explore the effects of plant density and harvest stage on quality and yield of *R. tanguticum*. Five density treatments (D1: 50 cm×10 cm, D2: 50 cm×20 cm, D3: 50 cm×30 cm, D4: 50 cm×40 cm, and D5: 50 cm×50 cm) and three harvest stages (green stage in early May, growing stage in middle July and wilting stage in early October) were chosen for this study from 2014 to 2016. Our results showed that: i) total biomass of dry plant decreased with plant density. Total dry biomass was the highest at growth stage and the lowest at green stage each year. Harvest index decreased with plant density in 2015 but slightly increased in 2016. Harvest index was the highest at wilting stage. ii) Yield per hectare increased significantly with plant density, and also with growth year and growth stage. iii) Growth year and stages either than plant density affected quality of *R. tanguticum.* iv) Yield of total anthraqunones have similar response to density treatments and harvest stages with biomass yield. In general, the best plant density for high yield of both biomass and total anthraqunones content was D1, but the best density for better appearance should be D4. Considered both quality and the economic production, wilting stage was suggested as the harvest season. 

**Keywords:** *R. tanguticum*; Plant density; Yield; Quality; Total anthraqunones

## Introduction

Roots and rhizomes of *Rheum tanguticum* Maxim. Ex Balf, *Rheum palmatum* L. and *Rheum officinale* Baill. are three original crude drugs of Rhubarb (Dahuang in Chinese) in the Pharmacopoeia of People's Republic of China (2015 edition) (National Pharmacopoeia Committee, 2015), which is a traditional Chinese medicine wildly used in the world as purgatives, heat-clearing drugs, and antibiotic drugs etc. It is so called "general" in traditional Chinese medicine because it usually plays an important role in curing emergency cases (Li, 2010). Hence, there are a great deal of studies has been made to explore the medical use of rhubarb (Wang and Ren, 2009; Miraj, 2016). Modern pharmacology researches have also proved that rhubarb could be used to treat constipation (Cirillo and Capasso, 2015), ischemic stroke (Lu *et al*., 2014) and Jaundice (Shan *et al*., 2017), and also have activities of anti-inflammatory (Bloom *et al*., 1959), anti-cancer (Huang *et al*., 2007; Shrimali *et al*., 2013; Li *et al*., 2014b), antineoplastic (Chen *et al*., 2014), protect liver and kidney, regulate blood sugar, prevent happens of hyperlipidemia, anti-HBV activities (Chen and Zhu, 2013; Qi, 2013) and molluscicide (Liu *et al*., 1997) etc.

Although most species of family *Rheum* have varies biological activities (Singh *et al*., 2016), *R. tanguticum* is regarded as the best one since its better efficacy in treatment experiences. *R. tanguticum* is a perennial plant in China, and mainly spread in Qinghai, Gansu, Sichuan, and east Tibetan. Wild plants usually grow at margins of forest, alpine valley and shrubs at the altitude ranging from 1600 m to 4200 m (Yang, 1991; Liu, 1997; Li, 1998). Most studies mainly focused on chemical component or comparison of *R. tanguticum* (Che *et al*., 2005; Cao and Tao, 2008; Li *et al*., 2008; Liu *et al*., 2013). And only few studies paid attention to cultivation management of *R. tanguticum* on the Qinghai-Tibetan plateau (Qi *et al*., 2015; Shen *et al*., 2017a). However, since the enormous requirement of *R. tanguticum* has caused excessive exploitation in China, storage of wild *R. tanguticum* has dramatically reduced according to wild resource investigation (Yang *et al*., 2001; Wang and Ren, 2009; Li *et al*., 2014a). Hence, cultivation of *R. tanguticum* should be expanded to fit the growing request of market.

Environmental factors and harvest stages have important effects on biomass accumulation and chemical

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compositions of *R. tanguticum.* Field management factors such as plant density and harvest stage may lead to different quality and yield of *R. tanguticum.* Plant density can influence yield and quality of many crops (Dong *et al*., 2010; Li *et al*., 2016; Zheng *et al*., 2016; Khan *et al*., 2017). Usually, at a lower density, larger biomass per plant could be harvested, while yield for hectare reduced. On the contrary, at a higher density, plant numbers significantly increased and thus yield per hectare increased, but larger biomass per plant could not be harvested at the same time. However, influence of density to plant usually depended on plant species (Severino *et al*., 2017), growth conditions and agricultural initiatives (Zhang *et al*., 2012). Few studies focus on the density effects on *R. tanguticum*. Hence, it is necessary to find out effects of plant density on yield and quality of *R. tanguticum*. Moreover, since plant at different growth stages have different compositions and component of metabolites (Brown *et al*., 1996). Another important factor has influences on yield and quality of many traditional Chinese medicine, such as *R. tanguticum* (Che *et al*., 2005), is harvest stage. In this study, yield and quality at three different growth stages will also discussed to choose the best harvest stage.

Specifically, yield of plant can be measured in two directions, yield for plant and yield for area. Besides, harvest index (HI) has also been brought in for a long time to evaluate yield potential in crops cultivation. HI was first coined by Donald (Donald, 1962; Donald, 1968) to describe ratio of “economic parts” to “biological parts”. The biological explanation of HI is allocation of photosynthate to different organs (Yuan and Guan, 1994). It is a widely used index to measure how much biomass transformed to economical yield in agriculture. Because while the total biological yield is constant, increasing HI is a good way to improve economic yield and thus raise economic returns (Hay, 1995). According to Chinese pharmacopoeia 2015 edition, predominant constituents on Rhubarb is total anthraqunones (TA), which content should be no less than 1.5% (National Pharmacopoeia Committee, 2015). Therefore, TA content was employed to evaluate quality of *R. tanguticum* in this study. Compositions of TA include rhein, chrysophanol, physcion, emodin and aloe-emodin in rhubarb.

In this study, specific objectives of this study were to explore effects of plant density and harvest stage on yield and quality of *R. tanguticum* in a field experiment. In this manuscript, effects of plant space on biomass, biomass allocation, yield and total anthraqunones contents were measured. And the best plant density was provided according to current research. As few studies has conducted on plant density and harvest stage of *Rh. tanguticum*, this study provide a foundational voice for further researches.

## Materials and Methods

### Experimental Site and Climatic Conditions

Field experiments were conducted in three consecutive growing years (2014, 2015 and 2016) at Donggou Township of Huzhu County, Qinghai Province, China (36°50′15″N, 101°57′06″E, and 2500m a.s.l.) with a typical plateau continental climate. The climate were characterized with relative dry and less rain, long illumination time, strong solar radiation, extremely cold winter, cool summer, and large temperature difference between day and night. Annual mean maximum and minimum temperature fluctuated from -0.7 to 31℃ and -23 to 16℃, respectively. The soil pH was 7.12 on average. And soil of experimental sites had 37.70 g/kg of organic matter, 1.77 g/kg of total nitrogen, 0.72 g/kg of total phosphorus, 21.67 g/kg of total potassium, and 0.99 g/kg of salt.

### Treatments and Experimental Design

**Treatment:** The experiment was a completely randomized block design with five densities, three replications blocks and three harvest stages each year. The five planting densities describes as D1 (50 cm× 10 cm), D2 (50 cm× 20 cm), D3 (50 cm× 30 cm), D4 (50 cm× 40 cm), and D5 (50 cm× 50 cm). Three harvest stages were set as green stage (early May), growth stage (middle July) and wilting stage (early October).

**Sowing and harvest:** Seeds of *R. tanguticum* were collected from Huzhu County, Qinghai, China and was identified by Professor Guoying Zhou. The voucher specimen was deposited in the herbarium of the Northwest Institute of Plateau Biology, Xining, Qinghai, China. Seedbeds of 1 m× 1 m were used to growing seeds in May 2013 at Donggou Township in Huzhu County, Qinghai, China. After one year's growth, selected germchit of basically homogeneous sizes transplant into different treatment blocks in May 2014. Average pot size is 120 $m^2$. Plants were harvested at wilting stage (early October) in 2014, and green stage (early May), growth stage (mid-July), and wilting stage (early October) in 2015 and 2016. For each replicate of five density levels, collected 10 plants from each block for experimental measurement and analysis. Above- and below-ground biomass were separated and measure the fresh weight. After drying the fresh roots to constant weight at dry oven of 45℃ (Liu, 2015), weight of dry materials were record for yield calculation.

### Materials and Reagents

Agilent 1260 system with a G1311A quat pump, G1315D DAD detector, G1329A autosampler, chromatography columns of 4.6×250 mm, 5 um, 100A, (Acchrom Technologies Co., Ltd., China) and Agilent HPLC software (Agilent, USA) were used for chromatographic analysis. Rhein, emodin, aloe-emodin, chrysophanol, physcion were purchased from the National Institutes for Food and Drug Control (Beijing, China). Water was purifies by using a Millipore Milli-Q system (Bedford, MA, USA). Acetonitrile and methanol of HPLC chromatographic grade was purchased from YuWang Group (Shandong, China). Other chemicals were analytical grade.

**Preparation of Sample Solution and Standard Solution**

Dried power of root sample (0.150 g) was accurately weighed and introduced into a 50 mL conical flask with cover, 25 mL methanol was accurately added into this flask, which was weighted and record. The flask was heated to reflux extraction for 1 h and then cooled to room temperature. Added methanol into the flask to the record weight and filtrated. 5 mL filtrate was taken into another flask and evaporated solvents using a rotary evaporator. The flask was added 8% v/v HCl (10 mL) and ultrasonically extracted for 2 min. Then, 10 mL chloroform was added into this flask. Heated the flask again for reflux extraction and cooled it to room temperature. After extracted with 10 mL chloroform for 3 times, combined the chloroform solutions and evaporate solvents with a rotary evaporator. Dissolved the residues with 10 mL methanol and filtered through a 0.45 μm organic membrane. All the operations were followed with *Chinese Pharmacopoeia.*

Reference compounds include emodin (lot code: 110756-200110), aloe-emodin (lot code: 110795-201007), rhein (lot code: 0757-200206), chrysophanol (lot code: 110796-201118) and physcion (lot code: 110758-201013). Standard solutions of emodin (95 μg/mL), aloe-emodin (140 μg/mL), rhein (171 μg/mL), chrysophanol (118 μg/mL) and physcion (104 μg/mL) were made by dissolving the chemicals in methanol. Injection volumes standard solutions of 2, 5, 10, 15, 20, 25, 30, 35, 40, 50 μL into the HPLC system to calibrate.

**Chromatographic Conditions**

The mobile phase A was 0.1% v/v phosphoric acid in UP water and mobile phase B was acetonitrile. The flow rate was 0.8 mL/min. The column temperature was 25°C. The linear gradient elution was as follows: 0-25 min, 50%-80% B; 25-30 min, 80% B. Chromatograms were recorded at 245 nm.

**Statistical Analysis**

Take into account of biological characteristics, calculate the yield by multiply dry mass of 10 plant as a yield of a special unit area for each density gradient, and divide the yield by the area of 10 pant of each density, and thus calculate yield per hectare at last.

The statistical analysis was performed by SPSS22.0. One-way ANOVA was used for significant test of variance. Significant difference was set at $P<0.05$ and using a Turkey test for multiple comparison when test of variance homogeneous past, and Kruskal-Wallis one-way ANOVA when test of variance homogeneous not past. Figures were performed by Sigma Plot 12.5.

## Result

**Biomass and Harvest Index**

One year old *R. tanguticum* was transplanted to the experimental site in May 2014. To make sure *R. tanguticum* had adapted to the new habitat, plants were harvest only in the October 2014, the wilting stage of *R. tanguticum*.

Average total dry biomass was 60.13 g/plant, 84.53 g/plant and146.64 g/plant respectively in 2014, 2015 and 2016 (Fig. 1). Total dry biomass increased with plant spacing in 2014 and 2015, but slightly decreased from D4 to D5 in 2016. Kruskal-Wallis one-way ANOVA suggest significant different between density treatments. The biggest biomass appeared at D4 of growth stage in 2016 (246.84 g/ plant). The biggest increase rate of dry biomass across all three stages among different densities appeared between D3 and D4, which increased 35.54% and 62.67% by average, respectively in 2015 and 2016. Total dry biomass increased from green to growth stage, but slightly decreased from growth to wilting stage since died back of above-ground parts. Average biomass of green, growth and wilting stage in 2015 and 2016 were 46.55 g/plant and 100.80 g/plant, 133.34 g/plant and178.02 g/plant and 101.08 g/plant and 164.48 g/plant, respectively.

At wilting stage, all above-ground parts died back totally, and thus HI of rhubarb was equal to 1 as showed in Fig. 1 (d, e and f). A decrease trend as the decrease of density at green and growth stages in 2015 was found, but an increased trends was found at green stage in 2016. One-way ANOVA confirmed significant difference between density treatments at green and growth stages, and Kruskal-Wallis ANOVA also suggested significant difference between D1 (0.60) and D4 (0.72) at green stage in 2016. In 2015, the highest HI was found at D1, which was 0.75 and 0.79 separately at green and growth stage. HI is the highest at wilting stage (1). HI was higher at growth stage (0.74) than at green stage (0.66) in 2015, but higher at green stage (0.66) than at growth stage (0.60) in 2016.

**Biomass Yield**

Yield of *R. tanguticum* in 2014 (a), 2015 (b) and 2016 (c) were shown in Fig. 2. One-way ANOVA suggested that plant density made an extremely significant effect ($p<0.001$) on yield of rhubarb. The highest yield at all harvest stages were obtained at D1, average yield at D1 was 12620.94 kg/ha. With the decreased of plant density, yield of *R. tanguticum* sharply decreased. Average yield at D1, D2, D3, D4 and D5 were 12620.94 kg/ha, 7060.42 kg/ha, 5837.85 kg/ha, 6259. 96 kg/ha and 4803.38 kg/ha. The difference between several densities was statistical significant at wilting stage in 2014, green and wilting stage in 2015 and all three stages in 2016.

Yield of rhubarb increased with the growth of *R. tanguticum.* Average yield of wilting stage in 2014, 2015 and 2016 are 5133.61 kg/ha, 8131.00 kg/ha and 12140.67 kg/ha, respectively. Besides, average yield of *R. tanguticum* also increased significantly during the growing season, and reached the maximum value at wilting stage (Fig. 2b and c).

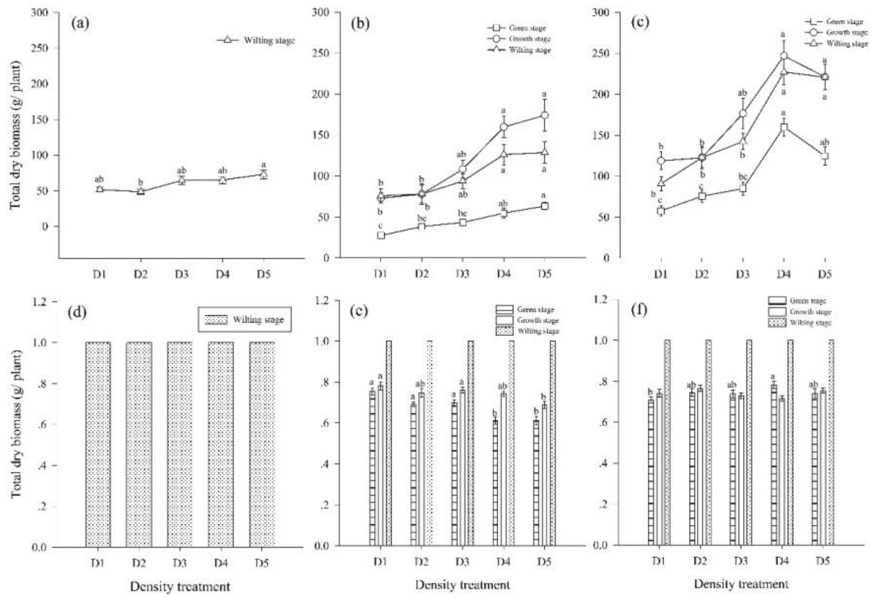


**Fig. 1:** Effect of plant density (D1, D2, D3, D4, and D5) on total dry biomass (a, c, e) and HI (b, d, f) of three year old *R. tanguticum* during 2014, 2015 and 2016. Data were expressed as mean ± standard error. Different letters indicate significant difference between density treatments ($p < 0.05$)

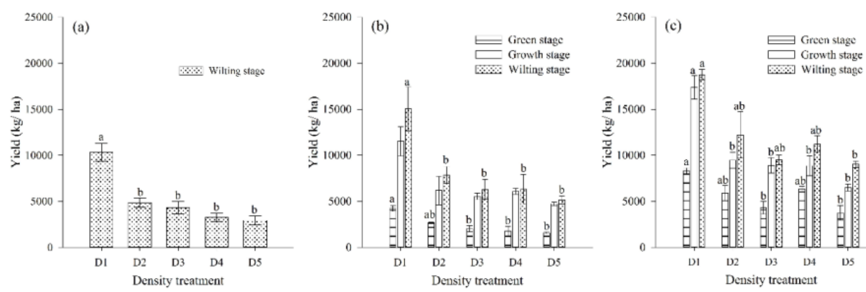


**Fig. 2:** Effect of plant density (D1, D2, D3, D4, and D5) and harvesting stage (green stage, growth stage and wilting stage) on yield of *Rh. tanguticum* during 2014 (a), 2015 (b) and 2016 (c). Data were expressed as mean ± standard error. Different letters indicate significant difference between density treatments ($p < 0.05$)

For the year 2015 and 2016, average yield of green, growth and wilting stages are 2326.64 kg/ha and 5714.82 kg/ha, 6852.76 kg/ha and 8131.00 kg/ha, 10215.94 kg/ha and 12140.67 kg/ha, respectively.

**Total Anthraqunones Contents**

With growth of *R. tanguticum*, TA increased (Fig. 3). Average content of TA was 0.87%, 1.88% and 2.35% separately at wilting stage in 2014, 2015 and 2016. Besides, a "U" shape appeared among different growth stages both in 2015 and 2016.Content of TA was higher at green stage (2.08% and 3.08% separately in 2015 and 2016) than at wilting stage (1.88% and 2.35% separately in 2015 and 2016), and growth stage (0.94% and 2.35% separately in 2015 and 2016). Significant difference was found between growth stages. And all pairwise comparisons revealed significant difference ($p$<0.05) between growth stage and the other two stages in 2015 and green stage and the other two stages in 2016.

Compare to growth year and growth stages, density treatments have smaller effect on TA content of *R. tanguticum*. Significant differences were found between density treatments at green and growth stage in 2015 and green and wilting stage in 2016. At green stage in 2015, average content of TA was the highest at density D2 (2.31%) and the lowest at D3 (1.92%). At the growth stage in 2015, average TA content was the highest at D1 (1.21%) and the lowest at D5 (0.68%). The highest TA content at green, growth and wilting stage were 3.37% (D3), 2.52% (D2) and 2.78% (D2), respectively. The lowest content value at three stages were 2.63% (D5), 2.15% (D3) and 2.12% (D3), respectively. Rhein, chrysophanol and physcion accounts for most of TA. Sum of percent of these three component account for 76.56% in average across all observed growth stages.

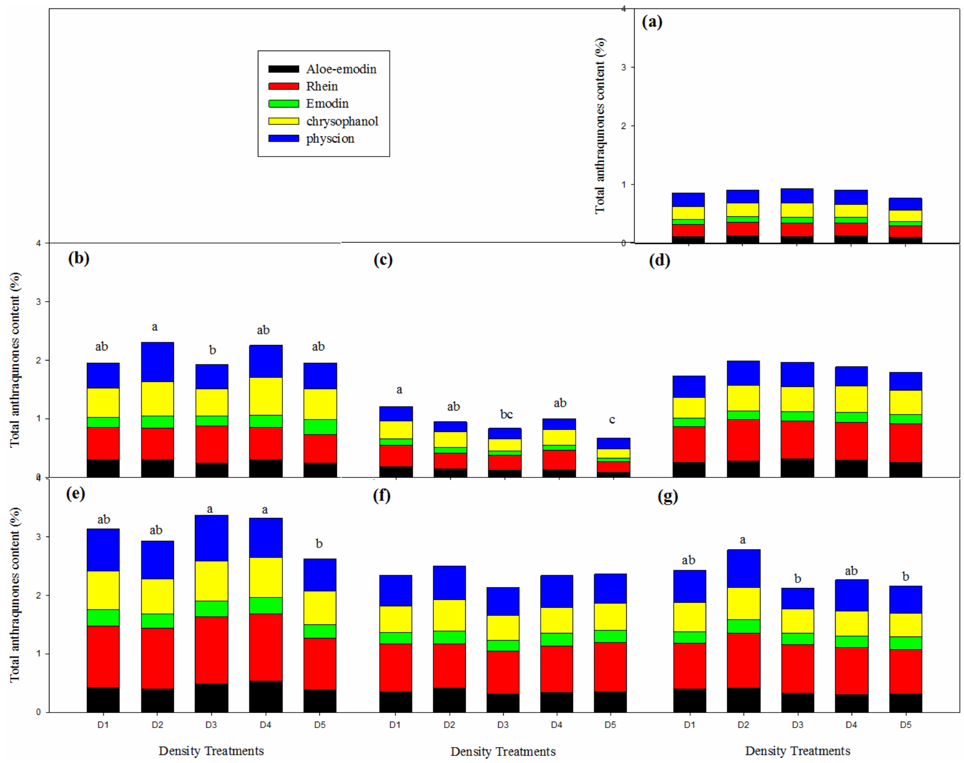


**Fig. 3:** Effect of plant density (D1, D2, D3, D4, and D5) on TA content of *Rh. Tanguticum* during 2014 (a), 2015 (b, c, d) and 2016 (e, f, g). Where (a) represent wilting stage in 2014, and (b), (c) and (d) represent green, growth and wilting stage in 2015, respectively. (e), (f) and (g) represent green, growth and wilting stage in 2016. Different colors represent different anthraqunones as expressed in figure. Different letters indicate significant difference of TA content between density treatments ($p < 0.05$)

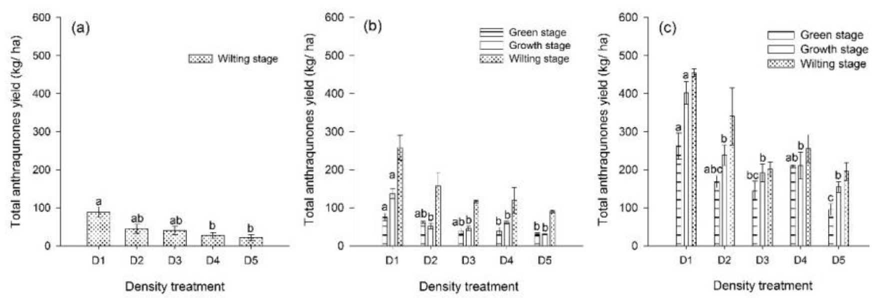


**Fig. 4:** Effect of plant density (D1, D2, D3, D4, and D5) and harvesting stage (green stage, growth stage and wilting stage) on TA yield in 2014 (a), 2015 (b) and 2016 (c). Data were expressed as mean ± standard error. Different letters indicate significant difference between density treatments ($p < 0.05$)

## Total Anthraqunones Yield

Yield of TA at five density treatments and three stages were showed in Fig. 4. One-way ANOVA revealed that significant effect had been made by plant density treatments. With the decrease of plant density (D1 to D5), yield of TA decreased. Average TA yield through all three years were 248.18 kg/ha, 156.85 kg/ha, 111.47 kg/ha, 131.79 kg/ha and 88.81kg/ha separately at D1, D2, D3, D4 and D5. Significant difference of TA yield between density treatments were found at wilting stage in 2014 (Fig. 4a), green and growth stage in 2015 (Fig. 4b) and green and growth stage in 2016 (Fig. 4c).

Yield of TA increased with the growing of *R. tanguticum*. Average TA yield at wilting stage in 2014, 2015 and 2016 were 44.60 kg/ha, 148.56 kg/ha and 289.72 kg/ha, respectively. An increase trend also found across the green to wilting stages in a year. In the year 2015 and 2016, average TA yield of green, growth and wilting stage were 47.42 kg/ha and 176.05 kg/ha, 66.51 kg/ha and

239.68 kg/ha, 148.56 kg/ha and 289.72 kg/ha, respectively. The whole trends of TA yield were the same with root dry mass yield. However, relative yield of TA at growth stage was lower than the relative biomass yield.

## Discussion

For cultivar industry of Chinese traditional medicine, both yield and quality are important (Romero-Trigueros *et al.*, 2017). In this study, biomass, HI index, biomass yield, TA content and TA yield were measured at 5 density levels and three harvest stages from 2014 to 2016. Our results revealed that biomass and yield were affected both by plant density and harvest stages, while quality of *R. tanguticum* was more affected by harvest stages.

Total biomass and biomass yield performed different response to plant density. Increasing plant density is regard as an important measurement to increase yield for many crops such as maize (Sangoi *et al*., 2002) and canola (Wang *et al*., 2015). Population density affect growth, development and population performance through its effect to light, temperature, $CO_2$ concentration, wind speed and microclimate, and eventually affect yield (Liu *et al*., 2000). Research find that plant density makes a significant effect on yield per unit area (Fig. 2). Yield of *R. tanguticum* decreased with the decrease of density. This result is opposite with maize (Abuzar *et al*., 2011), but similar with *Plukenetia volubilis* L. (Cai *et al*., 2013). Increasing yield with increasing density may result from the quantitative advantages of high density. However, a dramatic decrease of growth rate at density D1 was also glaring in this manuscript. Growth rate of yield at D1 density at wilting stage between 2015 and 2016 was 4.99 in average, which indicate that although amount of plant at D1 increase yield per hectare prominent, growth rate was largely restricted by too narrow space. Yield of plant per hectare at D2 density was getting closer to D1 density with the growth of *R. tanguticum*.

With the increase of plant spacing, average total biomass per plant increased constantly in the year 2014 and 2015 but reached a peak at D4 in 2016. The biggest value was at D5 in 2014 and 2015 but it was at D4 in 2016. Similar results were reported by Naser *et al*. (2013), which reveals that total dry weight per plant decreased with plant density at the first days but slightly increased between 4 plant dripper$^{-1}$ and 6 plant dripper$^{-1}$ at 100 days. Below-ground biomass was discussed by Shen *et al*. (2017b), who found that root biomass constantly decreased with increasing plant density except at green and growth stage in 2016. For the first two years, a larger growth spacing for each plant indicated that more sunlight energy and soil nutrient can be got for each plant. So more favorable conditions for growth lead to the biggest biomass appeal at D5. But competition appears among plants with the growth of *R. tanguticum*. Competition became increased and intenser with increasing density (Svanbäck and Bolnick, 2007). A moderate stress came from density D4 may motivate *R. tanguticum* to growth larger to

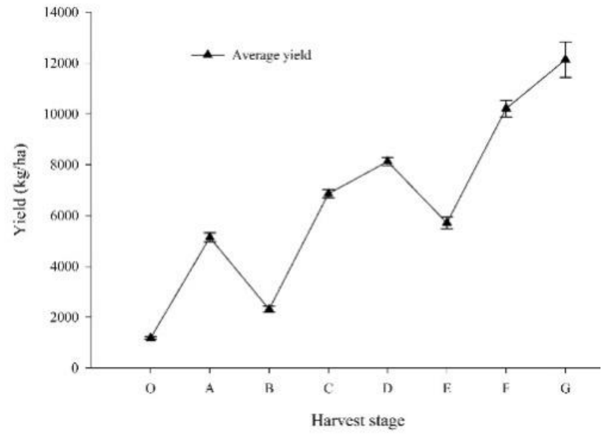


**Fig. 5:** Yield trends among different harvest stages. Average yield of 5 density treatments were expressed in this Fig, where O, A, B, C, D, E, F and G represent the original harvest yield in May 2014 and yield at wilting stage in 2014, green stage in 2015, growth stage in 2015 wilting stage in 2015, green stage in 2016, growth stage in 2016 and wilting stage in 2016, respectively

compete for more nutrients and especially light source. For that reason, both total dry biomass and root dry biomass reached the biggest value at D4 in 2016. Light distribution strongly response to plant density, and thus have effects on light use efficiency and biomass accumulation of plant (Zhu *et al*., 2010). Meanwhile, *R. tanguticum* has larger leaves, and canopy structure largely depends on plant density. Thus, the competition for light may larger than for soil nutrient. In the year 2015, biggest biomass of total plant and root all appear at D4 at green and growth stage, while root biomass at wilting stage was bigger in D5 than that at D4. Which indicate that the competition motivate *R. tanguticum* to accumulate more biomass may came from above-ground parts rather than below-ground parts.

Apparent seasonal dynamics of biomass and yield exists in this study (Fig. 1 and Fig. 5). At wilting stage, accumulation of biomass during growth season reached the biggest value within a year. But yield at green stage in the next year was lower than which at wilting stage in the previous year. Above-ground parts die back in winter, but below-ground parts may have respiration action during the whole winter (Janssens *et al*., 2004). Besides, sprout of new leaf before green stage also consumes the accumulations of *R. tanguticum* from the previous year (Hossain *et al*., 2001). Thus, the best harvest season for higher yield is wilting stage.

Although below-ground part of *R. tanguticum* is the main production for medical use, to understand effect of plant density on yield of *R. tanguticum*, make an observation on both above- and below-ground biomass in response to plant density is a key point. Because there is a mutual promotion relationship between below- and above-ground biomass of plant (Nie *et al*., 2016). HI was extensively used for explore the ratio that biological yield transplant to economical yield, which express the allocation of photosynthetic products to different organs (Yuan and Guan, 1994). In this study, HI was firstly brought in to discuss biomass relationship between above and below ground parts of *R. tanguticum*. This study reveals that HI decreases with the decreasing density in 2015, but keep constants in 2016. Which means that with the growth of *R. tanguticum*, plant density has declining effect on biomass allocation.

Plant density had no exact effect on TA in root of *R. tanguticum* in all three years of this research. But growth time and growth stage affect content of TA significant. Average content of TA in 2015 and 2016 all over the standard ruled by the Pharmacopoeia. Content of TA was much higher in 2016 than that in 2015 and 2014. Besides, an "U" shape of TA content by month (Fig. 3) was found and the similar with results of Shen *et al*. (2017a). This phenomenon revealed from current study just the same with the experiences of traditional herbalist doctors, which was that the optimal season for harvest *R. tanguticum* are the season before sprout of after die back of above-ground parts. Secondary metabolites product by secondary metabolism, which usually strengthened while some stresses existed. Environmental conditions such as light (Bieza and Lois, 2001; Cai *et al*., 2009; Hou *et al*., 2010), temperature (Zobayed and Kozai, 2005; Mori *et al*., 2007), soil water (Swigonska *et al*., 2014) and altitudes ( Katoch *et al*., 2011; Djerrad *et al*., 2015; Long *et al*., 2016) affect accumulation of secondary metabolisms. As a perennial plant mainly scattered among Qinghai-Tibet plateau, TA content was highest after winter and then before winter, which may be relevant to defense of cold stress of *R. tanguticum*.

Plant density usually make significant effect on yield but no uniform effect on metabolisms content. Combining both economic benefits and quality, yield of TA per hectare was surveyed. In this study, TA yield increased with plant density in all three years. Indicate that increasing plant density works quiet well in increase TA yield, although it's not a good manner for bigger root mass per plant. Harvesting at wilting stage would also provide higher TA yield due to the larger biomass yield at wilting stage. For production of TA, increasing plant density is definitely a good way to create more economic benefits. But for traditional Chinese medicine use, product appearance especially dry biomass or size of medicine is very important (Ji and Fang, 2014). Hence, there is no unified cultivation management, different plant densities should be chosen to fit different demands.

## Conclusion

In this study, five plant densities and three harvest stages were set to explore the best density and stage for high yield and quality production of *R. tanguticum.* Results showed that the plant density affect total dry biomass, yield and TA yield significantly and explicitly. For two, three and four year's old *R. tanguticum*, the best plant density for bigger dry biomass were D5, D5 and D4, respectively. But for both biomass yield per hectare and TA yield per hectare, the best plant density was D1. Harvesting stage also influence biomass and yield of *R. tanguticum* significantly. The best stage for both high biomass, yield and TA yield was wilting stage, besides, four years old plant was better than three and two years old *R. tanguticum.* Plant density also have significant effect on quality of *R. tanguticum*, but no exact density treatments were get for the best quality among all these stages. Compare with plant density, harvesting stages have more significant and explicit effect on quality of *R. tanguticum.* The highest quality of *R. tanguticum* was obtained at green stage, and then wilting stage, and growth stage product the lowest quality rhubarb. While, consider the much higher biomass and yield of rhubarb at wilting stage. Choosing wilting stage as the harvest stage is a better choice for economic product. In summary, density treatment D4 at wilting stage were suggest for better biomass yield per plant and quality of *R. tanguticum*, but density treatment D1 at wilting stage were suggest for the best yield of both biomass and TA per hectare and quality of *R. tanguticum.*

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