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ORIGINAL PAPER

The type, position and age effect on the cutting reproduction of *Picea crassifolia* and its rooting mechanism in the Qilian Mountains

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Abstract *Picea crassifolia* Kom, a perennial arbor species is recognized as one of the most adaptable plants found to date in Qilian Mountains. To explore the cutting reproduction technology of *P. crassifolia* and reveal its rooting mechanism, cuttings of *P. crassifolia* with different cutting types (softwood, hardwood and root), positions (top, upper, middle and bottom) and ages (7, 10, 15, 20, 25 year-old) were cultivated in a field experiment. One-year old softwood and hardwood cuttings were collected from 7-, 10-, 15-, 20-, and 25 year-old healthy ortets to analyze the changes from endogenous hormones and organic nutrients. Results indicate that the softwood cuttings (0.5–1.0 cm in diameter) from upper branches of 15 year-old ortets shows better growth performance by improving rooting indexes, including a significant increase in rooting rate and a decrease in basal rot rate. Concomitantly, increasing rooting quantity and root length also increased. It is noteworthy that the high rooting rate of *P. crassifolia* cuttings due to its ability to accumulate high concentrations of indole-3-acetic acid (IAA) and total carbon (TC) rather than abscisic acid (ABA) and total nitrogen (TN). The rooting rate was mainly regulated by the IAA/ABA and TC/TN ratio. In summary, our results suggest that the softwood cuttings (0.5–1.0 cm in diameter) from upper branches of 15 year-old *P. crassifolia* can be considered as an effective strategy to improve cutting rooting rate, and the IAA/ABA and TC/TN ratio was one of the main factors limiting the cutting rooting rate of *P. crassifolia*.

Keywords *Picea crassifolia* Kom · Type · Position and age effect · Cutting reproduction · Rooting rate · Hormones · Organic substances

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Introduction

Desertification has been recognized as a major environmental problem that inevitably triggers a loss of soil productivity and a progressive reduction of vegetation coverage (Gomes et al. 2003; Zhao et al. 2009). The Qilian Mountains is one of the major mountains of northwest China, and its unique forest ecosystem plays a crucial role in the construction of the ecological environment (Guo et al. 2003). However, the over and unreasonable use of forest resources has contributed to serious environmental degradation problems. Some plants, such as *Picea crassifolia* Kom and *Sabina przewalskii*, have evolved various adaptive mechanisms that allow them to survive and grow well in those environments. In facing the challenge of desertification, the physiological behaviors and adaptations involved in these species generate great interest (Zhang et al. 2009; Liu et al. 2012).

Picea crassifolia, a perennial arbor species, is recognized as one of the most adaptable plants found to date in Qilian Mountains (Duan 2012). This species is widely used to conserve soil and water in the barren mountain areas of northwest China. Meanwhile, the high productivity and excellent adaptability make *P. crassifolia* attractive as a garden ornamental species in local regions. At present, most researchers mainly concentrate on its growth and physiological characteristics, distribution patterns, and dynamics of population and sexual reproduction (Wang et al. 2009; Zhao et al. 2010; Meng et al. 2011).

However, due to the alpine climate and low seed vigor, the sexual reproduction of *P. crassifolia* is extremely restricted, leading to a short supply (Ji et al. 1996; Chen et al. 2007). Cutting reproduction has turned out to be a typical, convenient, and economical asexual reproduction technology, which has been widely used to clone *P. aspoerata* (Ma 1993; Wu 2008). Results showed that the early growth rate of *P. aspoerata* by cutting reproduction was significantly higher than that of seedlings (Browne et al. 1997; Bhardwa and Mlshra 1998), which cannot only keep the biological characteristics of ortets but also advance fruiting (Bengt 1981; Wang et al. 2011). The Research Institute of Forestry Genetics, Columbia, British has begun to produce mass clones of *Picea sitchenrsis*, which can resist the weevil parents (Talbert et al. 1993). The cutting result of *P. abies* showed that the rooting ability of different cutting types (length, diameter, position, age) was different (Bentzer 1988; Hannerz et al. 1999), and the rooting ability gradually declined with the increase of forest age (Kleinschmit and Schmidt 1977).

In China, many studies have been conducted on related themes of cutting and vegetative propagation of conifers (conventional, dwarf colorful, new superior, ornamental species, etc.) (Du and Zhou 2007; Huang and Huang 2011; Du and Jia 2012; Fang et al. 2013). The cutting results of *P. abies* suggest that the rooting numbers, total root length and the root-effect index of hardwood cuttings outnumbered softwood cuttings, and the cuttings length at >15 cm is the best (Jin et al. 2009). Wang (2008) analyzed the cutting propagation of four species of *Picea*, indicating that the cutting rooting rate was positively correlated with cutting length, breeding substrates, ages of ortets and hormones, and with the following order of *Picea likiangensis* > *Picea glauca* > *Picea mongolia* > *P. crassifolia.* Result also showed that softwood cuttings of *Pieea koraiensis* treated with different hormones and breeding substrates generated significant rooting rates. The hormones effect following order of IBA > NAA > IAA > ABT1 > CK and substrates effect order of forest soil > peat soil > perlite + peat soil (Liu et al. 2011).

Little is known about the systematic cutting reproduction of *P. crassifolia*, especially, the rooting mechanism. In this paper, we used *P. crassifolia* to explore the optimal cutting reproduction technology, and also discuss and analyze the endogenous hormones and organic nutrients in *P. crassifolia* cuttings to reveal its physiological and biochemical basis.

Materials and methods

Site description

The study area (Fig. 1) is located in Haxi Natural Protection Station (HNPS), Administration of Qilian Mountains Natural Nature Reserve, Gansu Province, Northwest China (37°16′–37°45′N, 102°01′–102°51′E; elevation: 2100–4140 m). The variation in temperature amplitude of and uneven distribution of monthly precipitation are the major meteorological features in this region. The annual average temperature in the area is 1.2 °C (extreme high temperature ranges from 28.5 to 32.4 °C in July and low temperature varies from −27.8 to −29.0 °C in January). On average, the annual mean sunshine hours is 1892 h, annual precipitation ranges from 401.9 to 632.3 mm, half of which comes in July and August and annual evaporation varies between 1041.2 and 1234.2 mm, and the relative humidity is about 58 % (Wang and Che 2012). The mountain grey brownish soil (elevation: 2400–3300 m) was the main distribution region of arbor forest (*P. crassifolia, S. przewalskii*). The *P. crassifolia* is distributed in the shade and half-shade slopes, which account for 24.7 % of the total area of water conservation forest and 75.7 % of the total area of arbor forest in Qilian Mountains (Peng et al. 2008).

Experimental design

Cutting type effect

Three cutting types (softwood: 0.3–0.5 cm and 0.5–1.0 cm, hardwood: more than 1.0 cm and root: 0.5–1.0 cm in diameter) and one cutting length (15 cm) were treated to select the most compatible cutting type of *P. crassifolia* (April to October 2009).

The softwood cuttings were semi-lignified branches with terminal buds, which were not affected by pests and diseases. Hardwood cuttings were strong branches with terminal buds, which were not affected by pests and

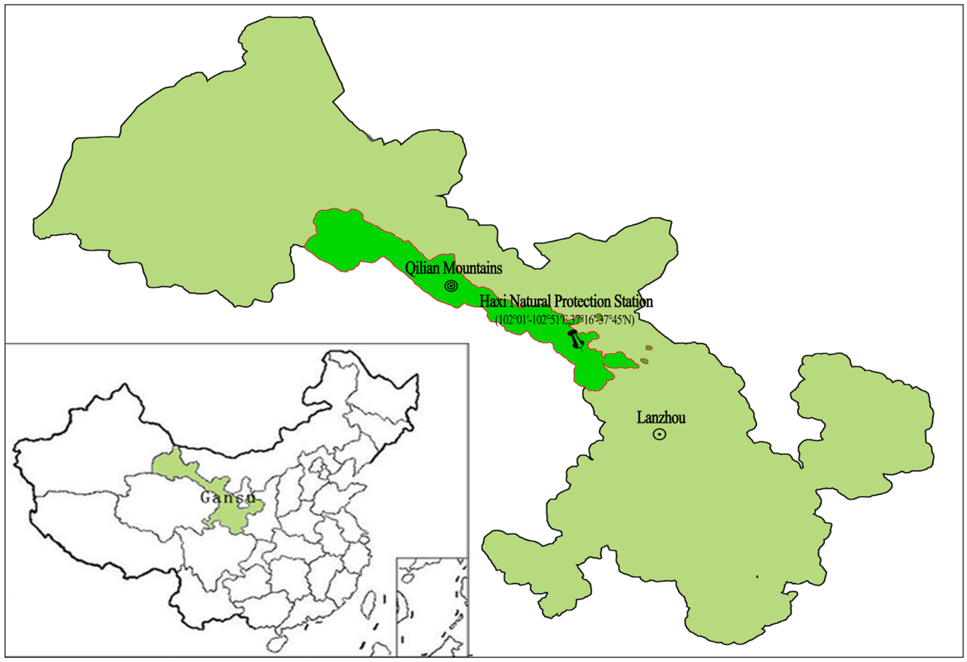

Fig. 1 Location of Haxi Natural Protection Station (HNPS), Administration of Qilian Mountains Natural Nature Reserve, Gansu Province, northwest China (37°16′–37°45′N, 102°01′–102°51′E)

diseases. Root cuttings (main or lateral roots) were collected from healthy plants where the above-mentioned *P. crassifolia* (10 years-old) was naturally distributed. Furthermore, the polarity of the cuttings was considered. The upper part of polarity should be flat, and the surface of cut should be 0.3–0.4 cm higher than the bud. The lower part should be a horseshoe-shaped oblique cut (in order to take root quickly) and the surface of cut should be 0.4–0.5 cm lower than the bud. To prevent cuttings from dehydrating by adverse circumstances, 50 cuttings were bundled together and packed in a plastic bag to keep moist.

The cutting experiment (field culture) was conducted at a greenhouse in Haxi Natural Protection Station (HNPS) and the nursery site was subdivided into 16 similar plots under the same site conditions. Each plot (1 m length × 1 m width) was surrounded by an isolated zone (0.2 m) and a seedbed was set up each plot (15 cm in height, 1 m in width) in order to reduce water loss and improve survival rate of the cutting. After all preparatory work has been done (the lower oblique cuts of cuttings were soaked for 24 h in water), cuttings were removed the needles and cut in each plot (10 cm in plant space; 15 cm in row space and 3–5 cm in cutting depth), and they were thoroughly watered.

The carbendazol (30–40 g/L) sprayed and resterilized on the seedbed once a week. Soil water content (SWC, 30 cm in depth) was maintained at 70 % of field water capacity (FWC) by weight; this value was maintained by irrigating with the 70 % FWC during the experimental period. Each treatment consisted of four plots containing 50 plants each. After the plants were harvested, 18 plants (survived, uniform plant) of each treatment were dug out and all plant tissues were washed three times with water for morphological analysis.

Cutting position and age effect

When the most compatible cutting type (softwood cuttings: 0.5–1.0 cm in diameter) was selected, we divided the whole tree into three equal parts (in addition to top branches) and defined as upper, middle, and bottom branches from top to bottom. Four positions (top, upper, middle and bottom) and one cutting length (15 cm) of 10 year-old healthy plants were treated to select the most compatible cutting position of *P. crassifolia* (May to October 2010). The detailed experimental process of the experiment was similar to that in different cutting positions of *P. crassifolia.* After the most compatible cutting position (softwood: 0.5–1.0 cm in diameter) and type (upper branches) were found, the cuttings were collected from 7-, 10-, 15-, 20-, and 25 year-old healthy ortets, which were treated to select the most compatible cutting age of *P. crassifolia* (May to October 2011). The detailed experimental process of the experiment was similar to that in different cutting types and positions of *P. crassifolia*.

Rooting mechanism of P. crassifolia cuttings

The experiment was conducted to determine whether the hormones or organic nutrients were one of the main factors limiting the cutting rooting rate of *P. crassifolia*. We did this by discussing and analyzing the effect of endogenous hormones (IAA, ABA and KT) and organic nutrients (TC and TN) (May to October 2012). One-year old softwood (0.5–1.0 cm in diameter) and hardwood (more than 1.0 cm in diameter) cuttings were collected from 7-, 10-, 15-, 20- and 25 year-old healthy ortets where the above mentioned *P. crassifolia* was naturally distributed. The phloem (20–30 g) of healthy softwood and hardwood cuttings was collected and placed in sealed plastic bags, and then taken to a laboratory for chemical analyses.

Measurements

Growth-related parameters

The rooting index of cuttings was determined, using routine methods after the growth stage ended. The mean root length (MRL) and total root length (TRL) were measured using a vernier caliper with 0.1 mm precision. The rooting rate (RR) was measured using the following formula: RR = the total numbers of rooted plants/the total numbers of cutted plants.

The root effect index (REI) was used to evaluate the rooting feature of various types of cuttings and the REI was estimated using the following formula: REI = (mean root length × the number of roots)/the total number of cuttings (Zhu and Wang 1991).

Endogenous hormones

The phloem (20–30 g) was collected according to the method described by Liang and Zhang (1997) and Buhtz et al. (2004). The endogenous hormones, including Indole-3-acetic acid (IAA); Abscisic Acid (ABA); and Kinetin (KT) were measured according to the method described by Li (2003). Briefly, the IAA, ABA, and KT were measured by the method of high-performance liquid chromatography (HPLC). The chromatographic column includes: Novapak C_{18} (0.4 cm × 15 cm); mobile phase: 40 % CH_3OH-15 %; CH_3CN-45 % H_2O (volume fraction); pH 4 (using H_3PO_4); speed: 0.7 m/L min; and detector: UV254 nm × 0.1AUFS.

Organic nutrients

The detailed collective method and process of phloem (20–30 g) were similar to the measurements of endogenous hormones. The organic nutrients, total carbon (TC) and nitrogen (TN), were measured by the method described in Guo et al. (2004). Briefly, the concentration of TC was measured by the method of sodium thiosulfate titration and the TN was measured by Kjeldahl determination.

Statistical analysis

All data was analyzed following SPSS program for Windows Version 13.0 (SPSS Inc., Chicago, IL, USA, 1975). Duncan's multiple range tests and one-way analysis of variance (ANOVA) were used to compare the differences among the treatments. The least significant difference (LSD) tests were performed to determine the significance of treatment means at $p < 0.05$.

Results

Effect of different cutting types on rooting characteristics of *P. crassifolia* (10 year-old)

The effect of different cutting types (softwood: 0.3–0.5 cm and 0.5–1.0 cm, hardwood: more than 1.0 cm and root: 0.5–1.0 cm in diameter) on the cutting characteristics (REI, RR, MRN, MRL, TRL, and BRR) of *P. crassifolia* have reached a significant level ($p < 0.05$), indicating that the different cutting types significantly affected the cutting rooting of *P. crassifolia* (Table 1). The rooting characteristics of softwood cuttings (0.5–1.0 cm in diameter) showed a better growth performance, and the RR was 28.1, 103.9, and 67.5 % higher than that of softwood (0.3–0.5 cm in diameter), hardwood (more than 1.0 cm in diameter) and root (0.5–1.0 cm in diameter) respectively The MRN were 1.4–2.3 times than that of the softwood (0.3–0.5 cm in diameter) and hardwood (more than 1.0 cm in diameter) cuttings. The BRR were 22.9, 37.3, and 74 % lower than that of softwood (0.3–0.5 cm in diameter), hardwood and root cuttings, respectively ($p < 0.05$).

Effect of different cutting positions on rooting characteristics of softwood cuttings (0.5–1.0 cm in diameter) of *P. crassifolia* (10 year-old)

The effects of different cutting positions (top, upper, middle and bottom) on the rooting characters (REI, RR, MRN, MRL, TRL and BRR) of softwood cuttings of *P. crassifolia* have reached significant level ($p < 0.05$), indicating that the different cutting positions also significantly affected the cutting rooting of softwood cuttings *of P. crassifolia* (Table 2). The rooting rate of softwood cuttings from top and upper branches showed a better growth performance, and the RR were 32.1 and 19.5, 49.8 and 37.2 % higher than that of middle and bottom branches respectively. The MRN were 72 and 36, 138.9, and 88.9 % higher than that of middle and bottom branches, and especially, the BRR were 23.8 and 10.8, 40.3 and 24.7 % lower than that of middle and bottom branches respectively ($p < 0.05$). However, although the cutting rooting rate of

Table 1 The growth performance of *P. crassifolia* cuttings treated with different cutting types (softwood, hardwood and root) and one cutting lengths (15 cm) for 6 months (May to October 2009)

Type (diameter)	REI	RR (%)	MRN (first-order)	MRL (cm)	TRL (cm)	BRR (%)
S 0.3–0.5 cm	2.4 ± 0.5b	52.7 ± 3.8b	5.0 ± 0.5b	4.7 ± 0.4b	93.6 ± 7.7b	47.4 ± 6.2c
S 0.5–1.0 cm	4.0 ± 0.7a	67.5 ± 5.2a	6.8 ± 0.4a	5.9 ± 0.6a	126.5 ± 9.2a	24.3 ± 3.0d
H More than 1.0 cm	0.6 ± 0.1c	33.1 ± 2.5c	2.9 ± 0.3c	3.0 ± 0.3c	56.1 ± 4.3c	61.6 ± 5.7b
R 0.5–1.0 cm	0	0	0	0	0	98.3 ± 7.1a

Values indicate the mean ± SD ($n = 6$). Different letters in each column indicate significant difference at $p < 0.05$ (Duncan test)

H hardwood, *S* softwood, *R* root, *REI* root effect index, *RR* rooting rate, *MRN* mean root number, *MRL* mean root length, *TRL* total root length, *BRR* basal rat rate (the same below)

Table 2 The growth performance of *P. crassifolia* cuttings treated with different cutting positions and one cutting lengths (15 cm) for 6 months (May to October 2010)

Position	REI	RR (%)	MRN (first-order)	MRL (cm)	TRL (cm)	BRR (%)
Top	6.2 ± 0.7d	86.3 ± 5.3a	8.6 ± 0.4a	7.2 ± 0.3a	141.3 ± 8.4a	10.3 ± 1.1a
Upper	4.0 ± 0.6b	73.7 ± 6.0b	6.8 ± 0.3b	5.9 ± 0.2b	125.5 ± 9.1b	23.3 ± 1.9c
Middle	2.3 ± 0.4c	54.2 ± 3.9c	5.0 ± 0.7c	4.6 ± 0.2c	101.3 ± 6.96c	34.1 ± 2.9b
Bottom	1.0 ± 0.2a	36.5 ± 4.6d	3.6 ± 0.6d	2.7 ± 0.3d	53.1 ± 7.8d	50.7 ± 5.6d

Values indicate the mean ± SD ($n = 6$). Different letters in each column indicate significant difference at $p < 0.05$ (Duncan test)

softwood cuttings from top branches was best, the tree structure was destroyed.

Effect of different ortet ages on rooting characteristics of softwood cuttings (upper branches) of *P. crassifolia*

The effects of different ortet ages (7-, 10-, 15-, 20-, and 25 year-old) on the rooting characters (REI, RR, MRN, MRL, TRL, and BRR) of softwood cuttings (upper branches) of *P. crassifolia* have reached a significant level ($p < 0.05$), indicating that the different ortet ages also significantly affected the cutting rooting of softwood cuttings (upper branches) of *P. crassifolia* (Table 3). The cutting rooting rate of softwood cuttings (upper branches) was increased first and then decreased significantly with the increasing age of *P. crassifolia*. The cutting rooting rate of 15-year old ortets was better than that of 7-, 10-, 20-, and 25 year-old ortets (Table 3). The RR of 15 year-old ortets were 38.1, 14.6, 28.5, and 48.8 %, higher than that of 7-, 10-, 20- and 25-year-old ortets. The MRN were 82.9, 41.5, 141.9, and 294.7 % higher than that of 7-, 10-, 20- and

Table 3 The growth performance of *P. crassifolia* cuttings treated with different ortet ages and one cutting lengths (15 cm) for 6 months (May to October 2011)

Ortet ages (years)	REI	RR (%)	MRN (first-order)	MRL (cm)	TRL (cm)	BRR (%)
7	1.4 ± 0.3b	39.7 ± 2.6c	4.1 ± 0.3c	3.5 ± 0.6c	87.6 ± 8.7b	57.3 ± 6.2c
10	2.6 ± 0.4a	62.6 ± 5.1b	5.3 ± 0.3b	5.0 ± 0.4b	116.5 ± 10.2a	33.1 ± 4.4b
15	4.3 ± 0.5c	77.2 ± 4.6a	7.5 ± 0.6a	6.3 ± 0.2a	138.8 ± 9.7a	20.4 ± 3.6a
20	0.8 ± 0.2d	48.7 ± 5.4d	3.1 ± 0.3d	2.6 ± 0.4d	63.4 ± 5.9d	46.6 ± 7.7d
25	0.3 ± 0.1d	28.4 ± 2.9e	1.9 ± 0.3e	1.4 ± 0.3e	49.4 ± 5.9d	67.8 ± 8.1de

Values indicate the mean ± SD ($n = 6$). Different letters in each column indicate significant difference at $p < 0.05$ (Duncan test)

Table 4 Relationship between endogenous hormones and rooting rate of hardwood and softwood cuttings of *P. crassifolia* at different ages (May to October 2012)

Ages (years)	IAA (μg kg^{-1})		ABA (μg kg^{-1})		KT (μg kg^{-1})		IAA/ABA		IAA/KT	
	H	S	H	S	H	S	H	S	H	S
7	663 ± 50a	573 ± 43a	245 ± 21e	27 ± 3e	338 ± 33a	267 ± 21a	2.7	21.2	1.96	2.15
10	604 ± 37ab	546 ± 41ab	312 ± 23cd	34 ± 4d	317 ± 29ab	243 ± 22ab	1.9	16.1	1.91	2.25
15	537 ± 39b	511 ± 34ab	357 ± 34c	43 ± 4c	296 ± 27ab	223 ± 18ab	1.5	11.9	1.81	2.29
20	474 ± 32c	415 ± 35c	441 ± 25b	55 ± 5b	261 ± 22b	187 ± 21b	1.1	7.5	1.82	2.22
25	322 ± 22d	236 ± 10d	572 ± 49a	69 ± 6a	224 ± 18bc	171 ± 17bc	0.6	3.4	1.44	1.38

Values indicate the mean ± SD ($n = 6$). Different letters in each column indicate significant difference at $p < 0.05$ (Duncan test)

IAA Indole-3-acetic acid, *ABA* Abscisic Acid, *KT* Kinetin

25-year old ortets. Finally, the BRR were 36.9, 12.7, 26.2, and 47.4 % lower than that of 7-, 10-, 20-, and 25 years-old ortets, respectively ($p < 0.05$).

Effect of endogenous hormones on rooting of hardwood and softwood cuttings of different ages of *P. crassifolia*

The effect of endogenous hormones on the rooting of softwood and hardwood cuttings of different ages of *P. crassifolia* (7-, 10-, 15-, 20-, and 25 year-old) were obvious (Table 4). The rooting rate of hardwood and softwood cuttings of different ages of *P. crassifolia* were positively correlated with the ortet ages and IAA, ABA, and KT content. The content of IAA and KT in hardwood and softwood cuttings significantly decreased, while the ABA was significantly larger with the increasing age of *P. crassifolia*. The content of IAA, ABA, and KT, and especially the ABA in hardwood cuttings, were significantly higher than that in the softwood cuttings with the increasing age of *P. crassifolia.* However, the IAA/ABA ratio has a positive effect (IAA/KT shows no obvious effect) on the rooting of hardwood and softwood cuttings with the increasing age of *P. crassifolia*. The IAA/ABA ratio in softwood cuttings of 7-, 10-,15-, 20- and 25 year-old *P. crassifolia* was average 7.34 times more than that of the hardwood cuttings respectively ($p < 0.05$). In short, the rooting rate of *P. crassifolia* cuttings was not decided by a single hormone but follows the IAA/ABA ratio. The bigger the ortets get, the smaller the IAA/ABA ratio has, and the lower rooting rate was either hardwood or softwood cuttings.

Effect of organic nutrients on rooting of hardwood and softwood cuttings of different ages of *P. crassifolia*

The effect of organic nutrients on the rooting of softwood and hardwood cuttings of different ages of *P. crassifolia* were also obvious (Fig. 2). The rooting rate of hardwood and softwood cuttings of different ages of *P. crassifolia* were positively correlated with the organic nutrient content, and the total carbon (TC) content in hardwood and softwood cuttings were decreased significantly, while the total nitrogen (TN) was showed the opposite trend with the increasing age of *P. crassifolia* (Fig. 2). However, the C/N ratio has a positive effect on the rooting of hardwood and softwood cuttings with the increasing age of *P. crassifolia*. The C/N ratio in softwood cuttings of 7-, 10-, 15-, 20- and 25 year-old *P. crassifolia* was 97.4, 119.5, 106.5, 82.7, and 103.8 % higher than that in hardwood cuttings. In short, the rooting rate of *P. crassifolia* cuttings was not decided by a single inclusion but by the C/N ratio. The bigger the ortets get, the smaller the C/N ratio becomes, and the lower the cuttings rate.

Discussion

The type, position, and age effect of *P. crassifolia* and its adaptation to alpine conditions

P. crassifolia is a perennial arbor species, and its growth condition is very complex resulting in its unique adaptation to alpine conditions. Our result showed that the amount of plant recruitment (*picea*) mainly relies on sexual reproduction to maintain its stability, and the growth performance of plants was extremely restricted due to their biological characteristics and habitat conditions which are far behind the asexual reproduction by cutting (Bengt 1981; Ma 1993).

In recent years, the sexual reproduction of *Picea* species is developing rapidly, and the application of various techniques in breeding (greenhouse seedling, container seedling, sand culture, etc.) shorten the breeding cycle of *Picea* species and improved the quality of seedlings (Cai 2010; Ji and Yao 2010; Kang et al. 2010). However, the sexual reproduction of *P. crassifolia* has a slow growth rate from

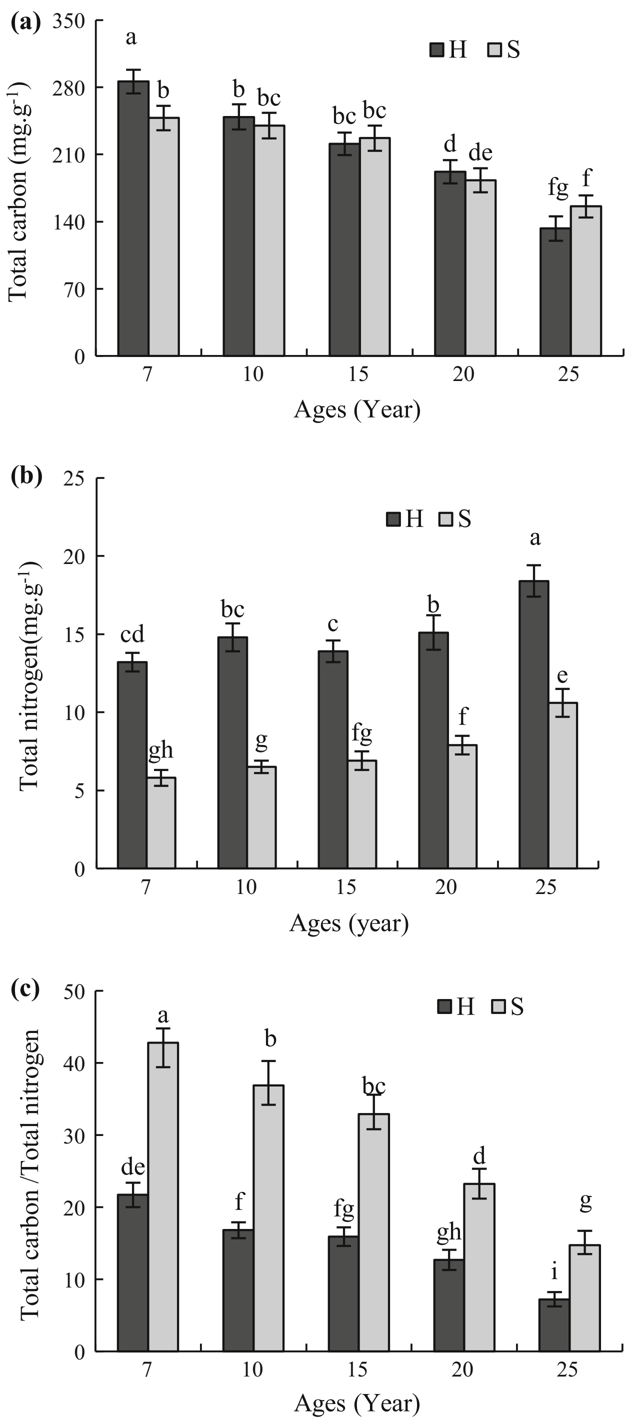

Fig. 2 Relationship between total carbon (**a**), total nitrogen (**b**), and total carbon/total nitrogen ratio (**c**) and rooting rate of hardwood (H) and softwood (S) cuttings of *P. crassifolia* at different ages (May to October 2012)

the seedling stage to the late fruit stage, a long time from first building a garden to seed production, seed yield instability, and low economic efficiency (Zhang 1992; Ji et al. 1996; Chen et al. 2007; Lei et al. 2012). Thus, the sexual reproduction of *P. crassifolia* may be virtually useless while the strong asexual reproductive performance plays an important role in mass production of *P. crassifolia* (Dormling and Kellerstram 1981; Bentzer 1988). In this study, the cutting reproduction of *P. crassifolia* has type, position and age effect, and the softwood cuttings (0.5–1.0 cm in diameter) from the upper branches of a 15 year-old ortet showed a better growth performance on the cutting rooting of *P. crassifolia* (Table 1, 2 and 3). This is because the rooting rate of softwood cuttings (0.5–1.0 cm in diameter) from upper branches of 15 year-old ortet produces strong metabolism, high endogenous hormone contents, and vigorous meristematic ability of cells (Talbert et al. 1993; Zhou 1995).

It is noteworthy that the age effect on the cutting reproduction of *P. crassifolia* has an obvious aging effect, reflected in the decreased cutting rate, declined rooting quality, and slowed growth (Table 3), which is mainly due to the progress of individual development caused by the physiological changes of the growth point (Ma 1993). Similar results have been reported for some other plants, such as *Picea koraiensis* (Nakai) (Zhao et al. 1997), *Cunninghamia lanceolata* (Lamb.) Hook (Shi et al. 1993; Guo 1997), *Pinus massoniana Lanb.* (P. sinensis Lamb) (Ji et al. 1998) and *Picea abies* Karsts (Talbert et al. 1993; Hu et al. 2008; Jin et al. 2009). From the countermeasures view of plants to adapt to environment, the asexual reproduction of *P. crassifolia* is the dominant choice, having many advantages including short breeding cycle, fast growth, strong resistance, and stable genetics. Researchers have successfully reproduced younger, stronger, and healthier cuttings by reducing the height of *P. abies* for asexual reproduction (Dormling and Kellerstram 1981; Clair et al. 1985). These results confirm that the evolutionary characteristics of asexual reproduction for *P. crassifolia* is an almost inevitable result under the environment stress. Each plant has its own complex physiological adaptive mechanisms that allow it to survive and grow well in a specific environment.

The effect of endogenesis hormones on the cutting rooting of *P. crassifolia*

In 1934, Holland physiologist (Went F.W.) published the seminal paper that shows that hormones have positive effect on the adventitious root formation of plants, and since then, many scholars found that softwood cuttings with high endogenesis hormone content take roots easily. The winter dormancy branch, and the rooting rate of cuttings with bud and leaves is higher than the cuttings getting rid of bud and leaves (Guo 1997). This is because the endogenesis hormone—produced by buds and leaves through polar transport and accumulation in the base of cuttings—played on significant role in rooting of cuttings.

Chen et al. (2007) showed that endogenesis hormone, 6-benzyl aminopurine (6BA), on the remaining buds after apical buds were removed, was more effective in boosting the growth and development of buds and branches in *P. crassifolia*.

Duan et al. (2009) showed that plant growth regulators (ABT1) can significantly promote the rooting of softwood cuttings with the order of *P. crassifolia* > *P. asperata* > *P. abies.* Jin et al. (2009) showed that cuttings of *P. abies* treated with different hormones generated significantly different rooting rates and with the effect following order of ABT1 > GGR > IBA > NAA. However, the number of roots generated, total rooting length, and the root effect index of the cuttings treated with IBA were better than those treated by other hormones. Moreover, being soaked two and half hourswith ABT1 at respective concentrations of 100 and 200 mg kg^{-1} proved to be the best in terms of rooting rates, total rooting length, and the root effect index.

Liu et al. (2011) indicated that the rooting rates, growth condition of seedlings, and rooting period of softwood cuttings of *P. koraiensis* obviously improved following treatment with 100 mg mL^{-1} IBA, compared with those treated by other hormones and following the order of IBA > NAA > IAA > ABT1 > CK. Similiar results have been reported on *P. abies* and some other *Picea* spicies such as *P. likiangensis, Picea glauca* and *Picea koraiensis* which showed that the rooting rate of cuttings was positively correlated with different hormone concentrations and immersion time (Hu et al. 2008; Huang et al. 2011; An et al. 2011; Liu et al. 2011; Hu et al. 2014).

Our results showed that endogenous hormones play important roles in rooting of *P. crassifolia* (Table 4): the IAA concentration and IAA/ABA ratio have positive correlation with cutting rooting rate, while the content of ABA has negative correlation with rooting rate (Table 4). This result shows that the low content of IAA (high content of ABA) made the IAA/ABA ratio very low, producing obviously inhibitory effects on the rooting of hardwood and softwood cuttings. These results are consistent with the results of Zheng (1991) and Zhang et al. (2004), which show that the IAA/ABA ratio plays an important role in cutting rooting of plants. Zhan et al. (2001) showed that the IAA and ABA contents play an important role in taking roots of *Betula platyphylla* Suk cuttings and the IAA/ABA ratio can be used as an important index to measure the cutting rooting ability. In short, the cutting rooting character of *P. crassifolia* is not decided by a hormone or inclusions, but by the synergetic effect of endogenous hormone and the inclusion. The higher IAA content and lower ABA content made the IAA/ABA ratio very high, which may be one of the main reason leading to a higher cutting rooting rate in softwood cuttings and a lower cutting rooting rate in hardwood cuttings.

The effect of organic nutrients on the cutting rooting of *P. crassifolia*

The C/N ratio reflects the nutritional utilization efficiency of plants and is an important supporter and regulator of plant-life processes. It is also one of the factors of the litter decomposition rate (Zhou et al. 2004, 2009). Healthy and developed cuttings are more likely to take root compared to cuttings with poor growth; clearly, the nutrient substances in cuttings may have a positive effect on the differentiation of the original body of roots (Guo 1997). Wang et al. (2011) analyzed the cutting propagation of three species of *Picea*, indicating that the physiological age and nutrient content of cuttings had significant correlation, and the nutrients, especially nitrogen (N) and potassium (K), in cuttings had great effects on the rooting of cuttings.

Our results showed that the cutting rooting rate of *P. crassifolia* correlated to the C/N ratio, the high content of TC and low content of TN made the C/N ratio very high, which plays an important role in the cutting rate of *P. crassifolia*, and the higher the C/N ratio is, the higher the cutting rooting rate gets. Moreover, the bigger the ortets get, the smaller the C/N ratio has, and the lower the cutting rooting rate was (Fig. 2). Guo et al. (2004) showed that the total sugar (TS), total nitrogen (TN), the ratio of TS/TN was positively related to cutting rooting of *Pinus bungeana.* The results of Guo et al. (2004) are consistent with our results, which showed that the TS and ratio of TS/TN were closely correlated to cutting rooting of *Pinus bungeana.* Moreover, many scholars considered that the nutrient substances in cuttings are not the critical factors on the formation of adventitious roots of cuttings. *Pinus bungeana* is more likely to take roots when the proportional relationship among carbohydrates, nitrogenous compounds, and endogenous hormones reaches a certain level (Guo 1997). The rooting of cuttings may be largely dependent on the degree of branch development (collection time of cuttings). We can consult the morphological and anatomical characteristics (the elasticity, color, lignified degree, and development degree of lenticels of branches, etc.) to carry out the cutting reproduction scientifically in a production setting.

Conclusions

Our results suggest that the softwood cuttings (0.5–1.0 cm in diameter) from upper branches of 15 year-old *P. crassifolia* can be considered as an effective strategy to promote cutting rooting rate and improve growth performance of roots. The IAA/ABA and TC/TN ratio in *P. crassifolia* cuttings was one of the main factors limiting the rooting rate of *P. crassifolia* cuttings. We determined that three factors—plant growth regulators, proper fertilization management of ortets, and bud-forcing treatment—can better improve the cutting rooting rate and growth performance of *P. crassifolia*. They are innovative and open up a new perspective for the improvement of *P. crassifolia* for biomass production in Qilian Mountains.

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