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EFFECT OF PROLONGED PHOTOPERIOD ON MORPHOLOGY, BIOMASS ACCUMULATION AND NUTRIENT UTILIZATION IN POST-TRANSPLANT *TAXUS CUSPIDATA* SEEDLINGS

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Abstract

Both threats and interest are the reasons for studying conservation and restoration programs of yew species. The seedling growth rate of slowly-growing species has been found to accelerate under prolonged photoperiod relative to that under the natural one, but the illumination effect has rarely been identified on yew species especially at their post-transplant stage. In the present study, one-year-old *Taxus cuspidata* seedlings were fed with exponential fertilization at the rate of 80 mg N/seedling under the prolonged photoperiod (18 h per day) with natural photoperiod (10.5 h per day) as the control in Northeast China. In the subsequent spring, seedlings were sampled to identify their post-transplant responses. Compared to the natural photoperiod, prolonged photoperiod increased seedling height, RCD, root length, and number of FOLR by 70% (p<0.0001), 30% (p=0.0037), 31% (p=0.0128), and 76% (P=0.0002), respectively. In addition, prolonged photoperiod increased dry mass in new shoot, old shoot, and root by 140%, 200%, and 153% (all P values <0.0001), respectively. In response to prolonged photoperiod, whole-plant nitrogen (N) and phosphorus (P) contents and utilizaitons decreased with the decline of N concentration in new shoot and P concentration in annual organ, respectively. Our results indicated that prolonged photoperiod did not promote, but stimulated, growth and biomass accumulation of transplanted *T. cuspidata* seedlings due to inherent decline of nutrient utilization for new growth.

Key words: Extended photoperiod, Intensive culture, Nutrient dilution, Exponential fertilization, Seedling quality.

Introduction

Yew (*Taxus* spp.) is an ancient gymnosperm and owns several congenital beneficial characteristics. The most famous "gift" from yew is paclitaxel which was firstly extracted from inner bark of *Taxus brevifolia* (Gohar *et al.*, 2015). Paclitaxel is a leading anti-cancer agent approved by the Food and Drug Administration (FDA) for the treatment of breast, ovarian, lung cancers as well as Kaposi's sarcoma (Han *et al.*, 2013). A series of toxoids have been identified from *T. cuspidata* (Kobayashi & Shigemori, 2002), *T. chinensis* (Sun *et al.*, 2009), *T. wallichiana* (Madhusudanan *et al.*, 2002), *T. baccata* (Glowniak *et al.*, 1999), etc. Another significant ability of yew is to accumulate radionuclides in annual organs without any sick symptoms (Minura *et al.*, 2014). Yew was also found to be able to survive and absorb formaldehyde (Yu *et al.*, 2015) as well. In northern Pakistan, branches of *T. wallichiana* are used to decorate welcome gates (Ahmed *et al.*, 2010). Due to these benefits, yew has been regionally afforded, e.g. by legal protection at top level in Europe Union (Perrin & Mitchell, 2013) and listed as endangered in China (Shaheen *et al.*, 2015). Therefore, both threats and interest are the reasons for studying conservation and restoration programs of yew in many regions of the world (Iszkulo *et al.*, 2014).

T. cuspidata is an endemic, evergreen, and dioecious species in Northeast China, Japan, Korea, and Russian Federation. Although this species was assessed in the Least Concern list (ver 3.1) (Anon., 2013), its exploitation was also justified to affect subpopulations in certain parts of its extensive distribution range and some decline may have occurred in areas with clear-felling of forests. Therefore, to clarify the cultural techniques would have some practical meaning for the exploitation of this species in parts of, at least, Eastern Asia. Because of slowly growing rate, the culture of *T. cuspidata* seedlings generally requests at least three years up before their quality meets the basic standard. Long cultural period has resulted in a significant restriction for their development. Thus, cuttings were suggested for propagation of *T. cuspidata* (Snyder, 1949; Wang *et al.*, 2012) whose growing rate at juvenile stage could be higher compared to their seedling stocks. However, the mature feature from the *T. cuspidata* cutting tended to be a large bush but not a tree. Instead, the ornamental tree feature can be easily obtained from their seedling stocks, which can also eliminate the potential loss of genetic diversity.

Light controls plant growth and development by facilitating photosynthesis and through a range of photoreceptor-mediated photomorphogenic processes (Devaney *et al.*, 2015). Artificially prolonged photoperiod could promote the seedling growth of some slowly growing species, such as *Pinus contoria* Dougl. (Wheeler, 1979), *Abies amabilis* [Dougl.] Forbes, *Tsuga mertensiana* [Bong.] Carr., *Picea glauca* [Moench], and *P. engelmannii* Parry (Arnott, 1979). Recently, new studies suggested that prolonged photoperiod can also promote biomass and nutrient accumulations in seedlings of sub-tropical tree species (Wei *et al.*, 2013; Zhu *et al.*, 2016a), but meanwhile may bring in the risks of in-coordinate morphology, nutrient dilution, and nutrient leaching (Zhu *et al.*, 2016a, b). These results were mainly from one-year studies which together indicated the potential degradation of seedling quality under the prolonged photoperiod. This cannot be confirmed unless post-transplant seedling performance was checked. About yew seedlings, however, the uncertainty of photoperiod influence is more significant than other species.

In the present study, *T. cuspidata* seedlings were cultured under contrasting photoperiods for one growing season and measured for growth and nutrient utilization after transplant. It was hypothesized that: post-transplant seedlings under the longer daily photoperiod during culture would have (i) greater morphology and biomass accumulation for all organs, and (ii) higher nitrogen (N) and phosphorus (P) contents and concentrations especially (iii) in newly growing organs.

Materials and Methods

Seedling materials and photoperiod treatment: *T. cuspidata* seeds were obtained from a nursery at Fengman District, Jilin City, Jilin Province, China (43˚46' N, 126˚36' E) in October 2011. Seeds were treated with standard protocols: sterilized by potassium permanganate (0.5%, w/w), soaked in water for 48 h, sand-stored underground for 18 months, and raised as bare-root seedlings for one year. On April 2014, 512 uniform-sized seedlings (height and root-collar diameter [RCD], 2 cm and 0.4 mm) were sampled and transported to the Laboratory (43˚59' N, 125˚23' E) and transplanted to plastic trays filled with peat (Zhuangmiao® Peat Sci. Ltd., Changchun, China) and perlite (3:1, v/v) at the density of 32 units (4 × 8) per 0.14 m^2 (length × width × height, 50 cm × 28 cm × 13 cm). All seedlings were transplanted into 16 trays and half of them were randomly placed to two rooms with natural and prolonged photoperiods. Prolonged photoperiod was supplied by plant growing lamps (Oudi Illumination Ltd., Huzhou City, Zhejiang Province, China) at the photosynthetic photon flux density (PPFD) of 400 µmol/m/s to lengthen the photoperiod to be 18 h per dayThe PPFD value during dark night was closed to the light saturation point (LSP) for natural yew regeneration (Devaney *et al.*, 2015).

Fertilization and transplant practice: Eight trays were randomly placed in either room as replicated groups (*n*=8). All seedlings were fertilized using an exponential fertilization model (Wei *et al.*, 2013) at a common rate of 80 mg N/seedling.. One unfertilized treatment was taken as the control. Fertilizers (20-20-20, Everris™, Geldermalsen, the Netherlands) were added accordingly once a week with sub-irrigation. Average temperature and relative humidity (RH) were measured to be 24.9˚C and 56.6%, respectively. Four months after fertilization commencement, photoperiods in both rooms were shortened to 6 h per day (10:00 a.m~16:00 p.m.) while substrate moisture was kept to be 50% to induce apical bud formation. . In early march 2015, ten seedlings were randomly sampled from one tray and transplanted into plastic pots (top diameter × bottom diameter × height, 8cm × 6cm × 6cm) filled with soils collected from five 60-year-old *P. tabuliformis* var. *mukdensis* plantations at Changchun (43˚47' N, 125˚28' E). For collected soils, bulk density was 1.31 g/cm^3, hydrolyzable N content was 93.89 mg/kg, available P content was 2.90 mg/kg, organic matter content was 2.38%, and pH was 5.5%. On 16 April 2015 when fully-expanded new shoots emerged, all transplanted seedlings were harvested and measured for height, RCD, root length, and number of first order lateral roots (FOLR). All seedlings were divided into parts of new shoot, old shoot (first-year needles and stem), and root. Each seedling part was oven-dried at 70ºC for 72 h and then measured for dry mass and determined for total N and P concentrations with kjeldah method and ICP-OES (Vista-MPX, Varian®, USA), respectively (Wei *et al.*, 2013; Zhu *et al.*, 2016a, b).

Statistical analysis: Nutrient absorption efficiency (*NAE*) was calculated as:

$$NAE = \text{Mass}_{\text{nutrients}} / \text{F}_{\text{nutrients}} \times 100\%$$

where, $\text{Mass}_{\text{nutrients}}$ is the total nutrient (N/P) content in the whole seedling, and $\text{F}_{\text{nutrients}}$ is the total dose of nutrients (N/P) added to the seedling (80 mg). Nutrient utilization efficiency index (*NUE*) was calculated with the formula modified from Hawkins (2007):

$$NUE = \text{Mass} / \text{C}_{\text{nutrients}}$$

where, Mass is the total dry accumulation amount of the whole seedling (g), and $\text{C}_{\text{nutreints}}$ is the average nutrient (N/P) concentration (%) for the whole seedling. Parameters (morphology, dry mass, and nutrient status) of ten transplanted seedlings were averaged for one replicated observation; hence, all data were calculated as two treatments (prolonged and natural photoperiods) with eight replicates. Analysis of variance (ANOVA) was conducted for all data based on the General Linear Model (GLM) procedure of SAS (9.0) (SAS Institute Inc., NC, United States). When significant effect was indicated, means were compared using Tukey test at 0.05 level. Vector analysis was employed to diagnose the comprehensive nutritional symptom for N and P in each seedling part according to the method by Salifu & Timmer (2003).

Results and Discussion

Seedling morphology: The photoperiod treatment had a significant effect on seedling morphology (Fig. 1). Compared to the natural photoperiod, prolonged photoperiod increased seedling height, RCD, root length, and number of FOLR by 70%, 30%, 31%, and 76%, respectively (Table 1). Because initial seedlings had grown for one year in the nursery, increase in the height was mostly contributed to by flushes under prolonged photoperiod and impacted a little by the flush after transplant. Significant height increment was also the robust result of prolonged photoperiod for *Podocarpus macrophyllus* and *Acer palmatum* seedlings (Wei *et al.*, 2013; Zhu *et al.*, 2016a, b), whose RCD and root morphology, however, showed null (Wei *et al.*, 2013; Zhu *et al.*, 2016a) or declined responses (Zhu *et al.*, 2016b).

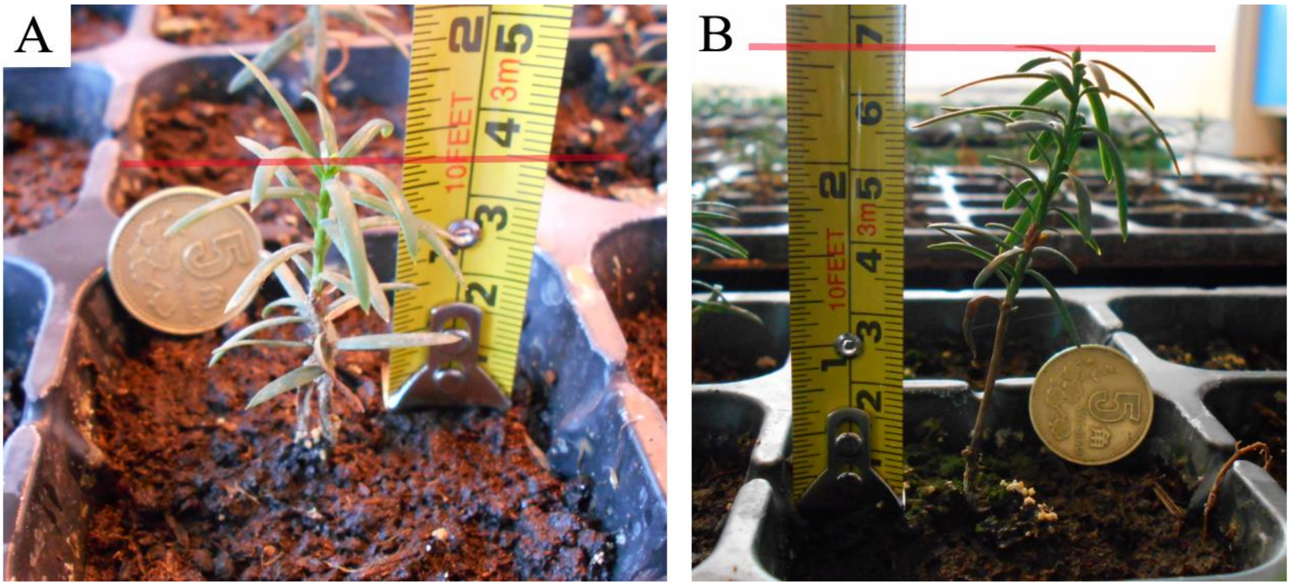

Fig. 1. Typical height of transplant *Taxus cuspidata* seedlings cultured under natural- (A) and prolonged-photoperiods (B).

Table 1. Effect of photoperiod treatment on morphology in *Taxus cuspidata* seedlings.

Treatment	Height (cm)	RCD (mm)	Root length (cm)	Number of FOLR
Prolonged photoperiod	6.99 ± 0.83a	1.24 ± 0.14a	13.36 ± 3.35a	13.00 ± 2.31a
Natural photoperiod	4.13 ± 0.22b	0.95 ± 0.16b	9.23 ± 1.56b	7.00 ± 1.59b

Note: Different letters in the same column indicate significant difference between treatments at 0.05 level. RCD, root-collar diameter. OILR, first order lateral roots

Table 2. Effect of photoperiod treatment on dry mass allocation in *Taxus cuspidata* seedlings.

Treatment	New shoot (g)	Old shoot (g)	Above-ground shoot (g)	Below-ground root (g)
Prolonged photoperiod	0.07 ± 0.02a	0.06 ± 0.01a	0.13 ± 0.02a	0.07 ± 0.01a
Natural photoperiod	0.03 ± 0.01b	0.02 ± 0.01b	0.05 ± 0.01b	0.03 ± 0.01b

Note: Different letters in the same column indicate significant difference between treatments at 0.05 level

Biomass accumulation and allocation: Compared to the natural photoperiod, prolonged photoperiod increased dry mass in new shoot, old shoot, and root by 140%, 200%, and 153%, respectively (Table 2). It was intersting that first-year photoperiod had a continuous promotion on biomass accumulation in second-year new shoot. Millard & Grelet (2010) indicated that build-up of non-structural carbohydrate (NSC) pools in trees represents accumulation (but not necessarily storage) of carbon (C) and is dependent upon the rate of current assimilation, but remobilization of C is sink-driven. Although root to shoot dry mass ratio (R/S) was not affected by the photoperiod treatment (data not shown), over 60% of biomass was detained in shoot part for most seedlings. Therefore, the greater biomass accumulation in *T. cuspidata* seedlings under prolonged photoperiodcan result from both stored C remobilization and continuous NSC accumulation because our seedlings did not suffer low temperature to induce deep dormancy during winter-time. However, it is reasonable to accept our first hypothesis.

Nutrient uptake and utilization: In contrast to the results of morphology and dry mass, seedlings under the prolonged photoperiod had low N concentration in new shoot and low P concentration in old organs (Table 3). Accordingly, both N and P contents declined in all seedling organs by prolonged photoperiod (Fig. 2). These results were quite different from former studies, where nutrient content was mostly increased due to the increase of biomass (Wei *et al.*, 2013; Zhu *et al.*, 2016a). Decline of N content in new shoot under prolonged photoperiod (Fig. 2A) resulted from decreased N concentration (Table 3), which was due to limited stored N for re-translocation (Fig. 3A). However, although the photoperiod-induced decline of N concentration but was not significant in old shoot and root, stimulated biomass accumulation resulted in N depletion by dilution in annual organs (Fig. 3B, C).

In the contrast to N results, decline of P content in new shoot under prolonged photoperiod (Fig. 2B) was not caused by P re-translocation due to unchanged P concentration therein; while decline of P concentration in annual organs may be due to the consumption of P for assimilation under prolonged photoperiod. P depletion can be related to fructose 6-phosphate (6-P) assimilation (Warren, 2011) and fungal growth (Lussenhop & Fogel, 1999). Additionally, according to vector direction and length, P showed more depletion than N under prolonged photoperiod. Thus, we cannot accept our second and third hypotheses.

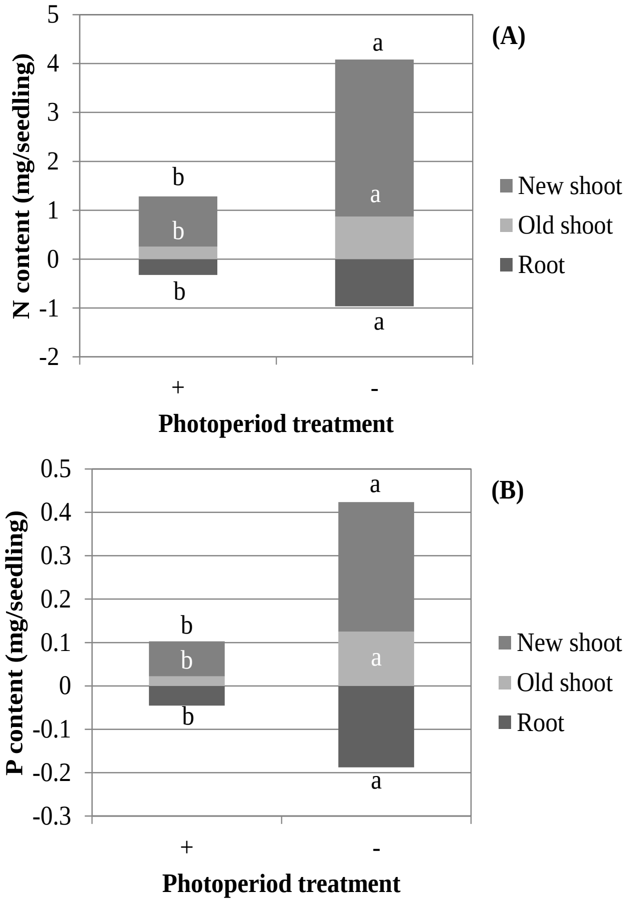

Fig. 2. N (A) and P contents (B) in parts of new shoot, old shoot, and root of *Taxus cuspidata* seedlings exposed to contrasting photoperiods.

Although both morphology and biomass accumulation were promoted by prolonged photoperiod, general nutrient uptake efficiency declined for both N and P (Table 4). Additionally, this low *NAE* resulted in insufficient nutrient uptake by *T. cuspidata* seedlings for intensive biomass accumulation under prolonged photoperiod, which resulted in low efficiency of nutrient utilization (Hawkins, 2007). The most possible reason to restrict *NAE* was the temperature, which may be too low in our study to satisfy root absorption. Otherwise, for an extremely slowly-growing species, such as *T. cuspidata* seedlings, the period of four months' nutrient delivery may be not long enough to demonstrate the photoperiod influence compared to those in Wheeler (1979), Salifu & Timmer (2003), Hawkins (2007), and Wei *et al*. (2013).

In conclusion, the present study revealed that prolonged photoperiod had significant effect to promote growth and biomass accumulation in post-transplant *T. cuspidata* seedlings. However, nutrient content declined in all seedling organs under prolonged photoperiod, where new shoots showed insufficient N re-translocation and annual organs of old shoot and root showed P depletion. Therefore, the promotion of prolonged photoperiod on growth and dry mass accumulationin *T. cuspidata* seedlings did not mean that their quality had also been enhanced due to the symptom of nutrient dilution and decreased utilizaiton. Future work is suggest to study the alleviation of nutrient dilution caused by prolonged photoperiod and to improve N and P utilizations in transplant *T. cuspidata* seedlings.

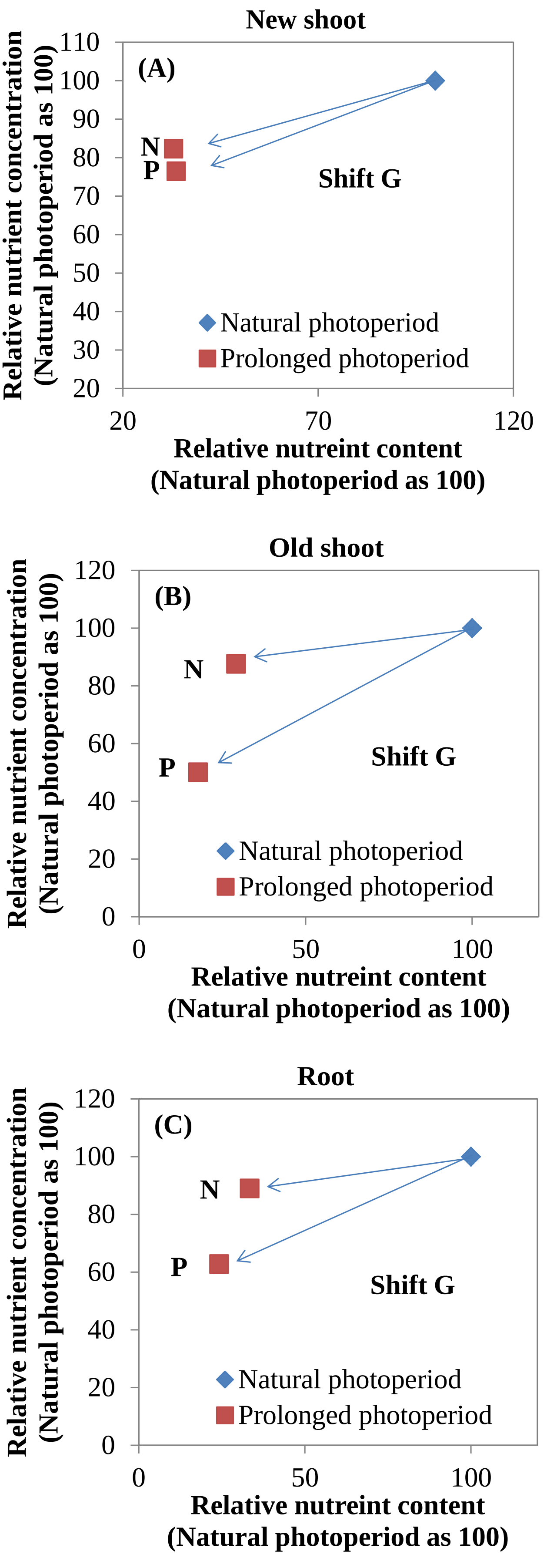

Fig. 3. Vector diagnosis of N and P contents and concentrations in parts of new shoot, old shoot, and root of *Taxus cuspidata* seedlings exposed to contrasting photoperiods.

Table 3. Effect of photoperiod treatment on N and P concentrations in *Taxus cuspidata* seedlings.

Treatment	New shoot (mg/g)	Old shoot (mg/g)	Below-ground root (mg/g)
		—N concentration—	
Prolonged photoperiod	27.22 ± 5.13b	12.64 ± 5.63a	11.97 ± 5.06a
Natural photoperiod	33.09 ± 2.06a	14.44 ± 5.32a	13.46 ± 3.65a
		—P concentration—	
Prolonged photoperiod	1.98 ± 0.44a	1.03 ± 0.27b	1.70 ± 0.70b
Natural photoperiod	2.59 ± 0.80a	2.06 ± 0.47a	2.70 ± 0.90a

Note: Different letters in the same column indicate significant difference between treatments at 0.05 level

Table 4. Effect of photoperiod treatment on efficient indices for N and P absorptions and utilizations in *Taxus cuspidata* seedlings.

Treatment	*NAE*		*NUE*	
	N	P	N	P
Prolonged photoperiod	1.69 ± 0.53b	0.16 ± 0.06b	47.19 ± 18.98b	0.49 ± 0.13b
Natural photoperiod	5.22 ± 1.15a	0.61 ± 0.08a	98.01 ± 14.63a	0.87 ± 0.27a
*P*r>*F*	50.35***	144.49***	29.23***	10.01**

Note: Different letters in the same column indicate significant difference between treatments at 0.05 level; **, $P<0.01$; ***, $p<0.001$

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