Effects of light on maca callus growth, shoot induction and its survival rate

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Abstract: Maca(*Lepidium meyenii*), belongs to the family of Brassicaceae, has high value of nutrition and medicine. In the process of micropropagation, light had remarkable influences on maca callus growth, shoot induction and survival. Green light and blue light not only made against maca callus growth but also inhibited shoot induction and survival. White light, red light and yellow light improved callus growth remarkably. Under these light conditions, the shoot induction rates were from 60% to 80%, and the shoot survival rates were from 29% to 36%. Appropriate prolonging of the time under light increased the shoot induction rate, and the appropriate photoperiod was 16h per day. But higher intensity of light decreased the survival rate of the shoots, and the appropriate light intensity was 24—41 $\mu mol/m^2 \cdot s$.

Key words: spectral quality; photoperiod; light intensity; maca; callus; shoot

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1 Introduction

Maca(*Lepidium meyenii*), a species belonging to the family of Brassicaceae, is mainly domesticated in the Andes Mountains in Peru where the climate is harsh and the altitude is 3 000 — 5 000 m. Maca was cultivated more than 2 000 years ago and used by Andean Indians as food and folk medicine to enhance fertility and sexual performance of men and women (Rea, 1992). Recently, the consumption of maca is increasing as its value has been reappraised. The research on maca was mainly about its compositions and pharmacological function. The tests of pharmacology showed that maca could enhance fertility (Kuo *et al.*, 2003) and sexual performance, improve sexual dysfunction (Zheng *et al.*, 2000), regulate incretion and nourish offspring (Canales *et al.*, 2000). Analyzing the compositions of maca hypocotyls showed that it was abundant in protein, essential amino acids, free fatty acids and many kinds of vitamin and minerals (Dini *et al.*, 1994), some secondary metabolite such as macaene and macamide (Zheng *et al.*, 2000), alkaloids (Cui *et al.*, 2003) and glucosinolate (Li *et al.*, 2001) were also identified in maca.

Studies on the tissue and cell culture of maca have been rarely reported. Our present work investigated the effects of light intensity, spectral quality and photoperiod on the maca callus growth, shoot induction and its survival rate.

2 Material and method

2.1 Plant material

Maca callus was induced in our lab as follow: Maca seeds were immersed in 70% (V/V) ethanol for 2 minutes, and rinsed three times with sterile distilled water. Subsequently, they were put into 2% (V/V) sodium hypochlorite solution for 20 minutes and rinsed three times with sterile distilled water.

For germination, disinfected seeds were placed in 150 mL conical flasks containing 50ml hormone-free MS (Murashige and Skoog's medium, 1962) medium

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(pH=5.85—5.90) and the medium was solidified by addition of 6 g/L agar. The seeds were cultured about 45 days under 16h light per day at 25±2 ℃. The light intensity of 24 μmol/m² was provided by white fluorescent lamps.

The roots of disinfected plantlets were cut into about 0.3 cm long and cultured in 100 mL conical flasks for 30 days under 16 h light (light intensity was 24 μmol/m²) at 25±2 ℃. Each flask contained 50 mL MS medium which supplemented with 1 mg/L 6-benzylaminopurine (6-BA), 0.5 mg/L naphthalene acetic acid (NAA) and 0.1 mg/L 2,4-dichlorophenoxy

Table 1　Main parameters of different lamps

Light spectral quality	White light (WL)	Red light (RL)	Yellow light (YL)	Green light (GL)	Blue light (BL)
Range of wavelength (nm)	380—780	610—715	530—790	480—670	380—560
Half-height bandwidth (nm)	—	15	90	50	85
Peak value of wavelength (nm)	—	658	600,632 (two peaks)	520	435

All lamp powers were 30 W

(2,4-D) and solidified by addition of 6 g/L agar. The calli were subcultured every 30 days on the same culture conditions as the former.

2.2 Effects of spectral quality and photoperiod on maca callus growth

In the tests of effects of spectral quality on maca callus growth, the main parameters of the lights provided by five kinds of lamps are listed in Table 1. The time of irradiation was 16 h per day. In the tests of photoperiod, the time of irradiation was 4 h, 8 h, 12 h and 16 h respectively and the light intensity was 24 μmol/m². s which was provided by fluorescent lamps. 0.6 g fresh weight calli were cultured on 50 mL MS medium (pH=5.85—5.90) in 100 mL conical flask for 30 days at 25±2 ℃ under different light conditions. The medium was supplemented with 0.5mg/L 6-BA, 0.25mg/L NAA and 0.1 mg/L 2,4-D, and solidified by addition of 6 g/L agar. Each culture condition had three repetitions. The callus fresh weights (FW) in each flask were weighted and then dried in an oven at 50 ℃ until their dry weights (DW) were constant.

2.3 Effects of spectral quality, photoperiod and light intensity on shoot induction

The conditions of spectral quality and photoperiod were the same as in the former experiments. In the tests of light intensity, the light intensities were 58 μmol/m² · s, 24 μmol/m² · s, 17 μmol/m² · s and 14 μmol/m² · s respectively. All induction temperatures were 25±2 ℃. The differentiation medium was MS supplemented with 2 mg/L 6-BA, 0.25 mg/L NAA. Each conical flask contained five pieces of calli, and each callus piece was about 0.7 cm³. After 25 days, the shoot induction rates were calculated.

2.4 Effects of spectral quality, photoperiod and light intensity on shoots survival rate

The shoots which were induced on differentiation media under white light at 25±2 ℃ for 25 days were cultured on subculture media under different light conditions at 15±1 ℃. The subculture medium was the same as differentiation medium. After 25 days, the well-growing shoots were counted and the shoot livabilities were calculated. The parameters of spectral quality and photoperiod were the same as the former. The light intensities were 96 μmol/m². s, 81 μmol/m². s, 53 μmol/m². s, 41 μmol/m². s and 24 μmol/m². s respectively.

3　Result

3.1 Effects of spectral quality and photoperiod on maca callus growth

In the tests of light influence on callus growth, different photoperiod had little distinct effect on callus growth (Table 2), but spectral quality could affect maca callus growth remarkably (Table 3). The biomass of maca calli decreased remarkably under blue light and green light than under white light, but they had no discrepancy under yellow light, red light and white light.

3.2 Effects of spectral quality, photoperiod and light intensity on shoot induction

In the experiments of light influence on shoot in-

duction, the shoot induction rates slightly increased as light intensities decreased (Fig. 1) and photoperiods prolonged (Fig. 2). Shoot induction rates were different when the *calli* cultured under different spectral qualities (Fig. 3). Under red light and yellow light, the shoot induction rates were 60% which resembled that under white light (80%). Green light and blue light made against shoot induction. Under these two lights respectively, the shoots almost died or grew abnormally, and the shoot induction rates were nearly zero.

Table 2 Effects of photoperiod on maca callus growth

Weight (g)	Photoperiod			
	4 h	8 h	12 h	16 h
FW	5.252±0.113	5.359±0.050	4.466±0.195	3.851±0.387
DW	0.406±0.034	0.357±0.035	0.346±0.012	0.344±0.089

Note: Indicates mean standard deviation

Table 3 Effects of spectral quality on maca callus growth

Weight (g)	Spectral quality				
	White light	Red light	Yellow light	Green light	Blue light
FW	4.134±0.100	3.490±0.305	3.800±0.311	3.500±0.103	4.045±0.253
DW	0.367±0.012	0.365±0.015	0.346±0.025	0.320±0.006**	0.313±0.011**

Note: Compared with white light, significant differences (t=0.01) are indicated with**; ± indicates mean standard deviation

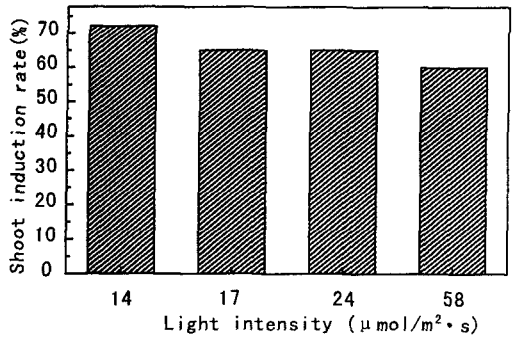

Fig. 1 Effects of light intensity on maca shoot induction rate

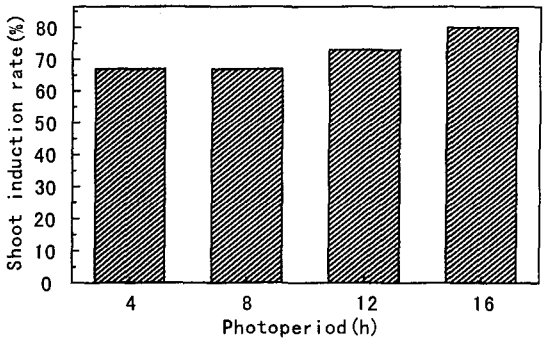

Fig. 2 Effects of photoperiod on maca shoot induction rate

3.3 Effects of spectral quality, photoperiod and light intensity on maca shoot survival rate

Spectral quality, light intensity and photoperiod all had remarkable effects on shoot survival rate. There was no discrepancy in shoot survival rate when the shoots were cultured under red light (29%) and white light (33%). Under yellow light, the shoot survival

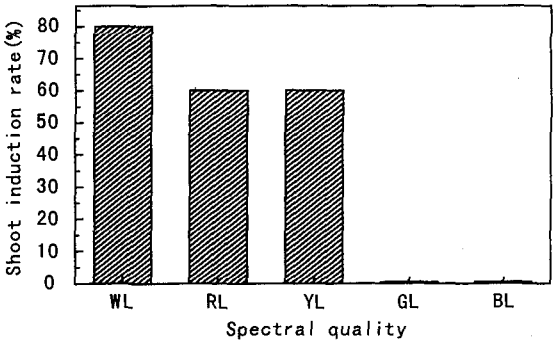

Fig. 3 Effects of spectral quality on maca shoot induction rate

rate (36%) was higher than those under other lights. Contrarily, the shoot survival rate was zero when they were cultured under green light and blue light (Fig. 4). In the tests of light intensity on shoot survival, the shoot survival rate decreased as light intensity increased. The highest survival rate of shoots was 33% when the light intensity was 24 $\mu mol/m^2 \cdot s$ and 41 $\mu mol/m^2 \cdot s$. The phenomenon showed that too strong light was harmful to shoots (Fig. 5). The results of photoperiod tests showed that shoot survival rates increased from 10% to 29% when the photoperiod was prolonged from 4 h to 16 h (Fig. 6). The optimized photoperiod to shoot survival was 16h per day.

4 Discuss

Light can influence plant growth through three aspects: light intensity, photoperiod and spectral quali-

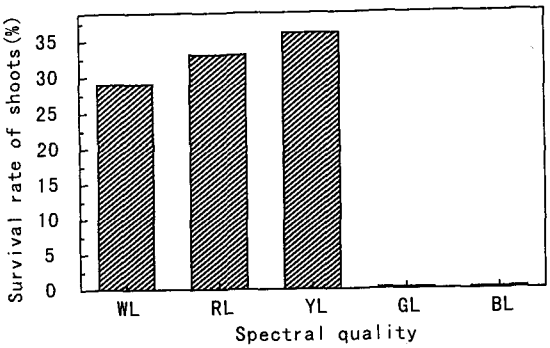

Fig. 4　Effects of spectral quality on maca shoot survival rate.

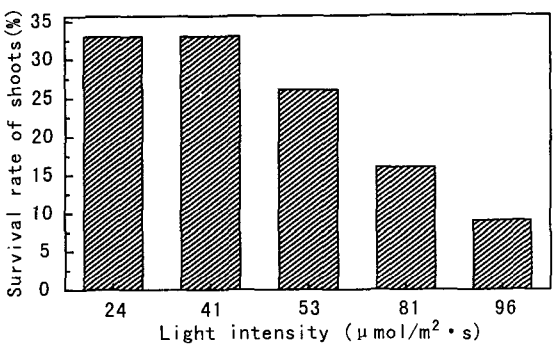

Fig. 5　Effects of light intensity on maca shoot survival rate.

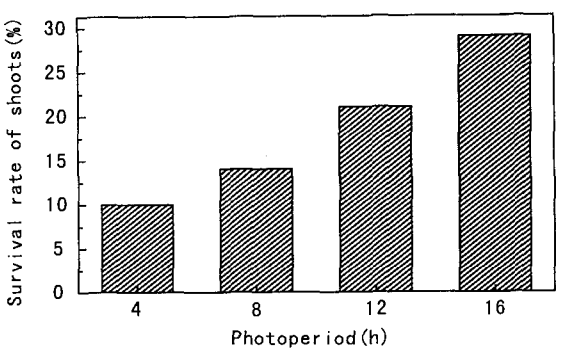

Fig. 6　Effects of photoperiod on maca shoot survival rate

ty. And the action of light on higher plants occurs mainly in two aspects. Firstly, light provides the energy source required by plants through photosynthesis. Secondly, light is a signal received by a photoreceptor to regulate the growth, differentiation and metabolism (Wang *et al.*, 2001). Different plants have different abilities to acclimatize to different light environments, and this ability is determined by genes in general (Lin *et al.*, 2004). Some studies showed that light could influence the plant cell and tissue growth and secondary metabolite biosynthesis. For example, spectral quality could influence the biomass of ginseng hairy roots and accumulation of ginsenoside (Yu *et al.*, 2005). Spectral quality and light intensity also influenced the callus growth of Cistanche deserticola and biosynthesis of phenylethanoid glycosides (Ouyang *et al.*, 2003).

In our study, photoperiod had little effect on maca callus growth. In the tests of spectral quality, the short wavelength lights such as green light and blue light inhibited the callus growth. Otherwise, white light and long wavelength lights such as red light and yellow light could improve callus growth. It was speculated that there should be some photoreceptors in maca callus which are more sensitive to longer wavelength lights. Just containing these photoreceptors, maca callus could utilize red light and yellow light more adequately for photosynthesis and to regulate the growth, differentiation and metabolism.

In the tests of shoot induction and shoot subculture, light intensity, photoperiod and spectral quality all could influence the induction rate and the survival rate of maca shoots. That the shoot induction rate and shoot survival rate under 16-h photoperiod were higher than under 4-h photoperiod respectively showed that enough light was essential to plant growth as well as to shoots. The reasons for that too much light was harmful to shoot induction and shoot survival rate might explain as follows. In photosynthetic organisms, even under optimal conditions, active oxygen species (AOS) such as the superoxide radical (O_2^-), H_2O_2 and hydroxyl radical (OH^-) can be produced as byproducts of photosynthesis. AOS plays a marked role in the dynamics of leaf senescence and damage of photosynthetic apparatus. Higher photooxidative stress means much more excessive excited energy is produced, and too much excessive excited energy could largely lead to production of AOS. The plant has developed several mechanisms to deal with excessive irradiance in order to avoid photodamage, but these mechanisms are dependant on leaf age (Jiang *et al.*, 2005). The light quality could influence the shoot induction rate and the

shoot livability. Under red light and yellow light, the shoot induction rates resembled that under white light. The survival rate was higher under yellow light than those under other lights. But under green light and blue light, the shoot induction rate and its survival rate were zero respectively. This phenomenon supports our estimation: there are some photoreceptors in maca callus which can utilize the long wavelength lights more effectively.

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光对马卡愈伤组织生长、丛生芽诱导和存活的影响

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摘 要：马卡属于十字花科独行菜属，具有极高的营养价值和药用价值。在快繁过程中，光对马卡愈伤组织生长，丛生芽的诱导和存活有显著的影响。绿光和蓝光既不利于愈伤组织的生长也不利于丛生芽的诱导和存活。白光、红光和黄光能明显促进愈伤组织的生长，在这些光照条件下丛生芽的诱导率为60%～80%，丛生芽存活率为29%～36%。适当延长光照时间可提高丛生芽的存活率，合适的光照时间为16 h/d。但是过强的光照可使丛生芽的存活率降低，合适的光照强度为24～41 μmol/m² · s。

关键词：光质；光周期；光强；马卡；愈伤组织；丛生芽