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Effects of light quality on growth, development, and photosynthesis of tobacco genetic resources in vitro

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Abstract

Light is an important factor affecting the growth of *in vitro*explant. Multiple cultures will increase costs and cause the variation of genes. In this study, effects of Yellow (Y), Red (R), Blue (B), 50%Y: 50%B, 50%Y: 50%R, 70%Y: 30%B, and 70%Y: 30%R were evaluated. Study results showed the light of 70%Y: 30%R effectively inhibited the growth of NC 628, and the plant quality was equivalent to W. With Burley 64, lights were unsuitable because they caused the ageing quickly. The 70%Y: 30%R light effectively inhibited the growth of 05 genetic resources, and plant quality was equivalent to W at the 100 DAC period. Next to 170 DAC, 70%Y: 30%R light inhibited the growth compared to the W, but caused the ageing of genetic resources faster than W. Light of Y and R effectively increased the leaf number and plant height, and B light sharply limited the growth of explants. Plant dry weight of 70%Y: 30%R and Y light was highest (0.47 - 0.5g), and lowest was B light (0.2g). Under Y, the leaf size was larger than other lights (1.9x1.99 cm), and the smallest leaf size belonged to B (2.85x1.73cm). Next time, we will research mixed light ratios to determine the suitable light for genetic resources.

1. Introduction

Light, including light intensity, quality, and photoperiod, was one of the factors influencing the growth and development of different plant species in test tubes (Hughes, 1981; Gupta et al., 2013; Chen Miao et al., 2023). Light was essential for various physiological processes in plants, such as photosynthesis, which affected plant growth and morphology (Gupta et al., 2013). Different light quality affected plant growth and development. LED lighting promoted the growth and physiological development of *Vanilla planifolia* seedlings and reduced lighting costs. LED of the Green (50%) and Red (50%) lengthened shoots and synthesize chlorophyll. Blue LED light stimulated shoot elongation, the number of roots formed and the number of leaves per shoot. White (W), red (R), blue (B) and a mix of B: R light did not differentially affect the number of shoots of *V. planifolia* after six weeks of the culture, but did differ in length, fresh and dry weight of shoots. R light inhibited the development of length, fresh and dry weight of shoots compared to W. The number of leaves/plant, root length, leaf area and number of stomata, R had the lowest value (M.A. Ramírez-Mosqueda L.G et al., 2017).

Light quality affected the morphology of tobacco plants. B light had the smallest leaf closing angle between the stem and petiole, so the plant was compact, while the leaf closing angle increased under yellow (Y), purple (P), (red) R, and green (G) light, and the plant had unconsolidated style. B light increased total soluble sugar and the reducing sugar content at R and G compared to W light. R and B light promoted photosynthesis and chlorophyll development, while Y and G light inhibited plant development (Yang, L.Y *et al*., 2017, Su et al., 2014).). G light strongly inhibited the plant height, Y and W light had the tallest plant height. The Y, B and G inhibited leaf length growth. Y light had a strong inhibitory effect on leaf width, then B and G, and finally W. Monochromatic light effectively inhibited plant growth, especially plant height, compared to W (Yang, L.Y et al., 2017). R light increased the stem length of some plant species, such as *chrysanthemum*, pepper, and *Phalaenopsis* (Schuerger et al. 1997, Kim et al. 2004, Shin et al. 2008), and increased the stem length of tobacco plants but less than W (Yang, L.Y et al., 2017). Leaf length and width decreased significantly under Y, G, and B. R light stimulated leaf length development compared to W, whereas B inhibited leaf length development. Lights of Y, P, R and G had a higher leaf length/width ratio than W, leading to long and narrow leaves (Yang, L.Y et al., 2017; Keet al., 2011). R light increasedChl and Car accumulation. On *Vanilla planifolia* orchids, FL, W, R, B, and B: R light did not significantly affect the number of shoots after 06 weeks of culture but had a significant effect on shoot length, especially was mixed light B: R. The fresh weight of W, B, and B: R light had little difference and the lowest was R. The dry weight of W was higher than R, B, B: R.

Light B stimulated root length growth, followed by B:R and R, and the shortest root length was W. B light had the most leaves; other lights (W, R and B: R) had the same number of leaves but were lower than the light of B. A mix of B: R light had the least number of roots, and other light types had the same number of roots. Root length did not differ with different light types. B light had a larger leaf area than light W, R and B: R. Light of B had the highest fresh weight of shoot and lowest of W, R and B: R. Dry weight of B was highest >then R > W and B: R. Light of R + B stimulated root growth and increased leaf area of *Doritaenopsis* tissue culture plants and carbohydrate biosynthesis more than monochromatic light. B: R light stimulated shoot growth and chlorophyll synthesis. B light stimulated the growth of shoot length, the number of roots formed and the number of leaves per shoot. The environmental survival rate of *in vitro* plants reached 100% in all types of light (M.A. Ramírez-Mosqueda et al., 2017). On Hyptismarrubioides plants *in vitro*, Y, R, and R light had greater shoot length than the control (W). R and G light increased the number of leaves on the plants. Fresh and dry weights under R were higher than the control (Rita Cassia N. Pedroso et al., 2017).

Research results of the Vietnam Tobacco Institute in 2013 with white (W), blue (B), red (R) light, light mix of 80% B and 20% R, 50% B and 50% R, 20% B and 80% R showed that B and R light inhibited the number of leaves/plant compared to W; The number of leaves was highest in mixed light of 50% B + 50% R. Monochromatic light inhibited the growth of leaves/plant. R light significantly inhibited plant height compared to W. In contrast, B light increased plant height compared to W. R light tended to reduce plant photosynthetic intensity compared to B and W. Root length did not differ statistically significant differences between varieties and light sources. Using LED lights in *in vitro* culture saved over 70% of energy, and the longevity of LED lights was 2.5 times that of fluorescent lights. Therefore, in this study, we investigate the influence of light quality on the growth and development, photosynthesis of tobacco genetic resources *in vitro*.

2. Materials and methods

2.1. Materials: The experiment was carried out on different types of light quality on 02 tobacco genetic sources in 2022, including of NC 628 (Flue-cured tobacco types) and Burley 64 (Brown tobacco types), and prospective light in 2023 with 05 tobacco genetic sources: Flue-cured tobaccos: NC628, NC 17, and K51E; and air brown tobaccos: Burley 64 and Tennessee that were took in the *in vitro* culture room. The types of light were used by LED lights: W(white), B (Blue), Y (Yellow), R (Red) and mixed light.

2.2. Methoids: The tobacco genetic resources were cultured on MS base medium (Murashige

Skoog, 1962). The experiments were completely randomized, with 12 replicates, and each replication was a triangle flask with one culture sample (Rami'rez-Mosqueda, M.A et al., 2017; Yang, L.Y et al., 2017). The culture samples were stored in the tissue culture room, with humidity of 60 - 65%, lighting time of 14 hours and room temperature of 22^{o}C ±2. Experimental treatments of the monochromatic colours and mixed light in 2022.

1. White lght (W - Control), with wavelength of 380 - 760nm.
2. Blue ligt (B): 420 - 480 nm.
3. Yellow light (Y): 570 - 630 nm.
4. Red light (R): 600 - 660 nm.
5. 50% Yellow and 50% Blue light (YB 50:50).
6. 50% Yellow and 50% Red (YR 50:50)
7. 70% Yellow and 30% Blue (YB 70:30).
8. 70% Yellow and 30% Red (YR 70:30).

Prospective light experiment: After determining the best suitable light in 2022, we performed the experiment of prospective light in 2023, with 05 tobacco genetic sources, including Flue-cured tobacco form (NC628, NC 17, and K51E) and brown tobacco form (Burley 64, Tennessee).

Monitoring targets:Plant height was measured from the surface of the MS medium to the top of growth, and plant length was from the surface of the medium to the tip of the highest leaf of the plant. Leaf length and width were measured in the middle leaves of *in vitro* plants. Leaf length was determined from the tip of the petiole adjacent to the stem to the leaf tip; leaf width from one leaf edge to the opposite leaf edge at the widest point. Root length was from the root collar to the growth tip of the longest root tip.Dry weight was treated at 60^{o}C in 6 hours until completely dry.

Calculate leaf area by using the weighing method (Hoang Minh Tan et al., 2000):

Leaf area: Cut 1cm^2 of fresh leaf area and weighed for mass P1 weight, then weighed total leaves of 05 plants to have P2. The leaf area was calculated by the formula: P2/P1.

Photosynthetic efficiency (PE): Took 05 random *in vitro* plants and washed the agar. Determining the area of leaf L1 as above, then dried the whole plants at 60^{o}C in 6 hours and weighed the weight of W1. The remaining other plants continue to grow for 90 days and performed as above to obtain L2 and W2. Photosynthetic efficiency (PE) was calculated by the formula:

$$PE = (P2-P1)/[1/2(L2+L1)xT]$$

The data was analyzed statistically to known the significance of the different parameters by statgraphics 19-X64 software.

3. Results and discussion

3.1. Effect of light quality on growth and development of tobacco genetic resources

3.1.1.Effect of light quality on growth and development of NC628 genetic resource

Table 1. Effect of light quality on leaf number, plant height and length, and aging speed of NC628 genetic resource

No	Light	50 DAC		70 DAC		80 DAC	140 DAC	
		No. leaf (leaf)	Plant height (cm)	No. leaf (leaf)	Plant height (cm)	Plant length (cm)	LTB	Aging speed
1	R	7.50 bc	2.16 d	10.76 c	3.15 e	6.6	LTB	Yellow foot leaves
2	Y	9.08 e	2.10 d	12.46 e	3.60 f	7.7		
3	B	6.17 a	1.65 b	8.76 a	1.91 a	4.6		
4	W	7.83 c	1.78 c	10.69 c	2.33 b	6.7	LTB	Green foot leaves
5	YR 70:30	7.83 c	1.46 a	10.84 c	2.05 a	5.3	-	

6	YB 70:30	8.33 d	1.80 c	11.53 d	2.91 d	7.6	LTB	Yellow foot leaves
7	RB 70:30	8.58 d	1.59 b	11.92 d	2.65 c	7.7		
8	YB 50:50	6.58 a	1.79 c	10.00 b	2.38 b	7.5		
9	RY 50:50	7.33 b	2.07 d	10.15 b	3.44 f	7.6		

Note: DAC: Day after culture,LTB: Leaf touch the vase's button, No. leaf: Number of leaves

Experimental results showed monochromatic and mixed lights affected the growth and development of the NC628 genetic resource, such as the number of leaves, plant height and length, and the ageing speed of the plant.

Leaf number: In some experimental lights, Y light strongly stimulated leaf number development of NC628 at the 50 - 70 DAC stage, with an average number of leaves from 9.08 to 12.46 leaves; then light of YB 70:30 and RB 70:30 (8.33 - 8.58 leaves); and B had the littlest number of leaves, only reaching 6.17 - 8.76 leaves. Compared with W light, the number of leaves of Y, YB 70:30 and RB 70:30 light was statistically significantly higher, with 95% confidence; the number of leaves of YR 70:30, R and W was equivalent and did not differ significantly; and of YB 50:50 and RY 50:50 was statistically significantly lower than W.

Plant height: Light of R, Y and RY 50:50 stimulated the strongest increase in plant height, reaching from 2.07 - 2.16 cm at 50 DAC and 3.15 - 3.6 cm with 70 DAC, and B light had the lowest plant height, ranging from 1.65 - 1.91 cm. Compared with W light, at the 50 DAC stage, plant height of R, Y and RY 50:50 was higher; YB 70:30 and YB 50:50 equivalent; and RB 70:30 and B was statistically significantly lower. In the stage of 70 DAC, Y and RY 50:50 light had the best plant height, respectively of 3.60 cm and 3.44 cm; then R (3.15cm), YB 70:30 (2.91cm) and RB 70:30 (2.65cm); and the lowest plant height belonged to light B, only reaching 1.91 cm. Compared with W light, plant height of Y, RY 50:50, R, YB 70:30, RB 70:30 was higher; of YB 50:50 equivalent; and the height of YR 70:30 and B was statistically significantly lower than W.

Plant length was one of three factors (*leaves touching the button, 2-3 foot leaves turning yellow, or culture nutrients being empty*) to determine the time to culture tobacco genetic resources. Among the 04 types of monochromatic light and 05 mixed lights, the plant length of Y, YB 70:30, RB 70:30, YB 50:50, and RY 50:50 light was the longest, fluctuating from 7.5 cm to 7.7 cm and higher than the W light (6.7 cm). The plant length of R was equivalent to the W, ranging from 6.6 - 6.7 cm. The plant length of YR 70:30 (5.3 cm) and B (4.6 cm) ranged 1.4 - 2.1 cm, and lower than the W light (6.7 cm).

Leaf touching the button and ageing speed of the plant: At the 140 DAC stage, most light treatments with the leaves touching the button of the triangle vase and base leaves turning yellow needed to be cultured. Particular the YR 70:30 light had a short plant length (5.3 cm), and the foot leaves did not turn yellow and were suitable for further research.

Thus, from the above research results, we determined the YR 70:30 light inhibiting the growth and development of NC 628 genetic resource, and the plant quality was equivalent to white light.

3.1.2.Effect of light quality on growth and development of Burley 64 genetic resource

Table2. Effect of light quality on leaf number, plant height and length, and aging speed of Burley 64 genetic resource

No	Light	50 DAC		70 DAC		110 DAC	
		No. leaf (leaf)	Plant height (cm)	No. leaf (leaf)	Plant height (cm)	Plant length (cm)	Plant aging speed
1	R	9,25 d	1,63 g	12,25 d	2,11 d	7,70	WYL
2	Y	9,17 d	1,45 f	13,33 e	2,96 e	6,75	
3	B	7,00 a	0,53 a	8,5 a	1,23 a	3,42	

4	W	7,67 b	0,75 b	10,83 b	1,68 c	4,96	GL
5	YR 70:30	7,66 b	0.86 d	10,83 b	1,50 b	3,80	YL
6	YB 70:30	8,42 c	0,74 bc	10,67 b	1,28 a	4,30	WYL
7	RB 70:30	8,66 c	1,16 e	11,41 c	1,68 c	5,03	
8	YB 50:50	8,25 c	0,76 bc	11,58 c	1,24 a	4,55	
9	RY 50:50	8,42 c	0,80 c	11,75 cd	1,45 b	4,67	

Note: WYL: White yellow foot leaves; YL: Yellow foot leaves; GL: Green foot leaves

Research results in Table 2 showed monochromatic and mixed lights affected the growth and development of the plant, such as the number of leaves, plant height and length, and ageing speed of the Burley 64 genetic source. Compared to the NC628 genetic source, Burley 64 was more sensitive to test lights.

Leaf number: Y and R light strongly increased the leaf number of Burley 64. At the stage of 50 DAC, Y and R light had the highest leaf number, fluctuating from 9.17 - 9. 25 leaves, statistically significantly higher than other experimental treatments; then YB 70:30, RB 70:30, YB 50:50, and RY 50:50 light had the same leaf number, but higher than W; Light of B had the lowest number of leaves, only reaching 7.0 leaves, and was lower than W. By 70 DAC, Y light had the highest number of leaves (13.33 leaves), then R (12.25 leaves) and the lowest was B light (8.5 leaves). The number of leaves of RB 70:30, YB 50:50, and RY 50:50 light was similar, lower than Y and R light, but significantly higher than the W, YR 70:30 and YB 70:30 light.

Plant height: Similar to the impact of light on the number of leaves, R and Y light strongly increased the plant height. At the 50DAC stage, R light had the tallest plant height, reaching 1.63 cm, followed by Y light (1.45 cm), and the lowest was B light (0.53 cm). YR 70:30, RB 70:30, and RY 50:50 light had a statistically significant higher plant height than the W, while YB 70:30 and W light had the same number of leaves. At the stage of 70 DAC, Y and R light had the tallest plant height, ranging from 2.11 - 2.96 cm; then RB 70:30 and W light (1.68 cm); Light of B, YB 70:30, and YB 50:50 light had the lowest height (1.23 - 1.28 cm). Light of RY 50:50 and YR 70:30 had significantly lower plant height than W, but higher than B, YB 70:30, and YB 50:50.

Plant length: Similar to plant height, Y and R light had the highest plant length, ranging from 6.75 - 7.70 cm; B light strongly inhibited the plant length compared to W.

Plant quality was an important indicator to determine the time to culture genetic resources when foot leaves turned yellow/leaves touched the vase button. Monitoring results in the period of 110 DAC showed all *in vitro* explants had base leaves turning yellow-white under the test lights. In contrast, the white light (W) had the foot leaves remaining green.

Thus, experimental light types were unsuitable for storing Burley tobacco genetic resources, so needing to continue to research the proper light for Burley tobacco, because experimental light types caused the ageing of genetic resources faster than white light (W).

3.1.3.Effect of light quality on some growth and development and accumulating dry matter of NC628

Table 3.Effect of light quality on growth of root, leaf size, and accumulating dry matter of NC628

Light	Root length (cm)	Fresh root mass (g)	Dry root mass (g)	Fresh mass of plant (g)	Dry mass of plant (g)	Leaf size (cm)		Rate ofL/ W	Leaf area (cm^2)	PE (g/m^2/day /night
						L	W			
R	12,25	2,39	0,09	8,43	0,33	3,90 cd	1,99 b	1,99	37,98	6,51E-5
Y	13,0	2,14	0,09	10,16	0,47	4,12 d	2,35 cd	1,75	60,34	6,76E-5

B	4,45	0,21	0,01	3,79	0,20	2,85 a	1,73 a	1,65	24,38	5,02E-5
W	17,50	1,76	0,08	8,79	0,37	3,53 bc	1,99 b	1,77	54,61	6,23E-5
YR 70:30	8,50	1,24	0,07	8,50	0,37	4,08 d	2,53 d	1,61	73,48	4,63E-5
YB 70:30	11,0	1,65	0,09	10,67	0,43	4,08 d	2,26 c	1,81	56,18	6,03E-5
RB 70:30	11,25	1,97	0,09	11,95	0,50	3,40 b	2,01 b	1,69	48,09	6,51E-5

Note: L: Length; W: Width, PE: Photosynthetic efficiency

Light quality was an important factor affecting the growth, development and dry matter accumulation of tobacco genetic resources, specifically:

Root length: Among the experimental lights, white light (W) had the longest root length, up to 17.5 cm, followed by Y (13.0 cm), R: 12.25 cm and the lowest under light of B, with a root length of 4.45 cm. YR 70:30 light had root length, fresh and dry root mass more than B, and lower than other lights, leading to low fresh and dry matter accumulation of plant, despite leaf size and leaf area being the largest.

Root fresh and dry weight: *In vitro* plants under R and Y light gave the biggest fresh root weight, reaching 2.39 g and 2.14 g, respectively, then RB 70:30 (1.97 g), W (1.76 g), and the lowest was B light (0.21 g). Light of YR 70:30 and YB 70:30 had fresh root weight lower than W, but more than B. About the dry root weight, the light of R, Y, YB 70:30 and RB 70:30 had the highest dry mass, reaching 0.09 g, then W: 0.08 g, YR 70:30 was 0.7 g, and the lowest was B light (0.01g).

The fresh and dry weight of the plant (including roots, stems and leaves): Tobacco genetic sources under RB 70:30 light had the highest fresh weight, reaching 11.95 g; then YB 70:30 and Y light, ranging from 10.16 - 10.67 g; W: 8.79 g; and light of B had the lowest fresh weight (3.79 g). Similar to fresh weight, RB 70:30 had the highest dry weight, reaching 0.5 g, YB 70:30 and Y light, with 0.43 g and 0.47 g, respectively, and the lowest wasB light (0.2 g). In the experimental lights, the light of YB 70:30, RB 70:30 and Y effectively increased the fresh/dry weight, while other experimental lights reduced the dry weight of plants compared to W.

Leaf length and width: Light of Y, R, YR 70:30 and YB 70:30 the strongest increased in leaf length, ranging from 3.90 - 4.12 cm, and statistically significant longer than W (3.53 cm) from 0.37 - 0.59 cm; followed by RB 70:30 and W, with leaf length of 3.4 cm and 3.53 cm, respectively; and lowest was B light (2.85 cm). For leaf width, *in vitro* plants under YR 70:30 and Y light had the largest leaf width, reaching 2.53 cm and 2.35 cm, respectively; then to YB 70:30 was 2.26 cm; and B light had the narrowest leaf width (1.73 cm). Leaf width under R, RB 70:30, and W light were similar and did not differ statistically. On the contrast, the leaf width of YR 70:30, Y, B and YB 70:30 light had significant differences compared to W.

The length/width ratio was one of the important elements for leaf and plant morphology. *In vitro* plants under B and YR 70:30 light had the lowest leaf L/W ratio, ranging from 1.61 - 1.65, and had large leaf shapes, whereas, under R light, the leaves of the genetic source had long leaf shapes. Regarding plant morphology: Under R light, the stem internodes between the leaves were long, the leaf closing angle was normal, and the canopy was even; light of Y, B, YB 70:30 and BR 70:30: uneven canopy, closed leaf angle with large trunk and horizontal leaves; YR 70:30: even canopy and normal leaf closure angle.

Light quality played a major decisive role in a plant's ability to accumulate fresh/dry matter. Accumulating fresh/dry matter also depended on the area of photosynthetic leaves. Study result showed B light was a sharp inhibition on plant growth and development, leading to the lowest photosynthetic leaf area and dry matter accumulation. Light of RB 70:30 had leaf size and photosynthetic leaf area (48.09 cm2) lower than W, YB 70:30, Y and YR 70:30 light (54.64 - 73.48 cm2), but the ability to accumulate highest nutritional value, with fresh/dry weight of the plant of 11.95/0.5g, while other lights had larger leaf area but had lower fresh/dry weight, ranging from 8.5 - 10.67/0.37- 0.47g.

Tobacco genetic resources preserved under YR 70:30 light had the largest leaf area, reaching 73.48 cm^2, then Y (60.34 cm^2), and the lowest was blue light (B), with a leaf area of 24.38 cm^2.The ability of plants to accumulate dry matter depended not only on the photosynthetic area but also on the quality of light. Data in Table 3 showed that Y light had the highest photosynthetic efficiency, reaching 6.76E-5 g/m^2day/night, followed by RB 70:30 and R (6.51E-5 g/m^2day/night), and the lowest was B (5.02E-5 g/m^2day/night) and YR 70:30 (4.63E-5 g/m^2day/night). Compared with W light, Y, R and RB 70:30 light had higher photosynthetic efficiency than W, and other lights had lower PE than W. Photosynthetic efficiency of genetic resources that did not depend much on leaf area and leaf size affected mainly on the quality of light. For example, the leaf area of YR 70:30 was the largest, reaching 73.48 cm^2, but the photosynthetic efficiency was the lowest (4. 63E-5 g/m^2day/night).

3.2. Test the promise medium of YR 70:30 on 05 tobacco genetic resources

The results of testing light types in 2022 identified a promising light of 70%Y + 30%R (YR 70:30) was suitable for preserving slow growth. In 2023, we tested 05 tobacco genetic resources on YR 70:30, consisting of Flue-cured tobacco (NC628, NC17, K51E) and air brown tobacco (Burley 64 and Tennessee). The test results are shown in Table 4.

Table 4. Impacts of YR 70:30 light on growth of 05 tobacco genetic resources

Tobacco genetic resource	Treatment	80 DAC		100 DAC		Plant length 130DAC	Plant aging speed		
		No. leaf (leaf)	Plant height (cm)	No. leaf (leaf)	Plant height (cm)		130 DAC	140 DAC	170 DAC
NC628	YR 70:30	13.33 a	2.05 a	15.75 a	2.58 a	5.9 a	GL	GL	WYL
	Control (W)	13.25 a	2.23 b	16.0 a	2.81 b	6.6 b	GL	GL	GL
Burley 64	YR 70:30	13.58 a	2.54 a	19.25 a	3.39 a	8.2 a	LGVN	WYL	WYL
	Control (W)	15.83 b	2.88 b	19.0 a	3.94 b	11.6 b	GL		
Tennesse	YR 70:30	8.25 a	1.26 a	11.16 a	1.27 a	3.4 a	LGV	YL	YL
	Control (W)	8.25 a	1.40 b	11.75 a	1.60 b	4.4 b	GL		
NC 17	YR 70:30	8.50 a	1.75 a	10.0 a	2.88 a	7.8 a	GL	GL	WYL
	Control (W)	9.41 b	2.34 b	11.58 b	3.22 b	9.3 b	GL	GL	GL
K51E	YR 70:30	10.5 a	2.40 a	12.75 a	3.45 a	8.5 a	GL	GL	LGV
	Control (W)	10.91 b	2.71 b	12.66 a	4.30 b	11.15 b	GL	GL	GL

Note: WYL: White yellow foot leaves; YL: Yellow foot leaves; GL: Green foot leaves

Prospective light of YR 70:30 affected the growth and development of 5 tobacco genetic sources, such as the number of leaves, height and length, and ageing speed of plants over storage time.

Number of leaves per plant: Data in Table 4 showed that the prospective light of YR 70:30 did not have much impact on the development of the number of leaves/plant of 5 tobacco genetic resources. Specifically, at the 80 DAC stage, the number of leaves of NC628, Tennesse, NC17 and K51E genetic resources under the prospective light and the control was not statistically different, only under 0.91 leaves; only Burley 64 had the highest difference leaf numbers between the light of YR 70:30 and W, up to 2.25 leaves. At the 100 DAC stage, the leaf number of the genetic sources under YR 70:30 and W light was nearly equal, and there were no statistically significant differences. Only the NC17 had a leaf number more than the control light (W), about 1.58 leaves, and the difference was statistically significant with 95% confidence.

Plant height: The light of YR 70:30 effectively inhibited the growth of 5 significant tobacco genetic sources compared to W. At the stage of 80 DAC, the plant height of 5 genetic sources under YR 70:30 light fluctuated from 1.26 - 2.54 cm, statistically significantly lower than W (1.4 - 2.88 cm) from 0.14 - 0.59 cm. Similarly, the plant height of YR 70:30 (1.27 - 3.45 cm) was lower than W light (1.6 - 4.3 cm) from 0.23 - 0.85 cm at the stage 100 DAC. Among the experimental genetic resources, Tennessehad the shortest plant height at YR 70:30 light, reaching 1.27 cm, and statistically significantly lower than W light. The K51E had the highest plant height, reaching 3.45 cm and was statistically significantly lower with W (4.30 cm).

Plant length was one of three requirements to determine the culture of genetic resources to the next cycle. The length of the plant under YR 70:30 light was significantly lower than the control (W).

Specifically, at the 130 DAC stage, under the condition of YR 70:30 light, the plant length ranged from 3.4 to 8.5 cm, significantly lower than the control (4.4 - 11.6 cm). The plant length of Tennesse was the lowest, while K51E and Burley 64 had the highest plant length at both YR 70:30 and W light. Under W light conditions, the length of K51E and Burley 64 *in vitro* plants ranged from 11.15 - 11.6 cm, with over 50% of explants having leaves touching the bottom of the flask and needing to be cultured, while under the YR 70:30 had plant heights of 8.2 - 8.5 cm.

Level of plant ageing: At the 130DAC period, the flue-cured tobacco genetic resources grew and developed normally, and the foot leaves remained green compared to the control. Meanwhile, Burley tobacco genetic resources, such as Tennesse and Burley 64, were too sensitive to YR 70:30, and the foot leaves aged quickly and turned yellow, compared to W light with base leaves not turning yellow. At the 140DAC stage, the Burley 64 aged fast in both YR 70:30 and W light, with yellow-white foot leaves, then Tennesse, with yellow foot leaves. With other genetic sources, plants grew normally. At the 170DAC stage, under prospective light, flue-cured tobacco genetic resources, such as NC628, NC 17 and K51E, had a faster ageing speed than W because most of the *in vitro* plants' foot leaves turned yellow to white-yellow, compared to the W light the foot leaves remained green.

Results of testing 05 tobacco gene sources on the prospective light of YR 70:30 showed the storage time at 100DAC the effect of inhibiting plant height growth and ageing speed was equivalent to the control. From 130DAC onwards, the ageing speed of plants of YR 70:30 light gradually increased and was most evident at 170DAC. The burley genetic resource was more sensitive to YR 70:30 light than flue-cured tobacco. The study results of the prospective light at the stage of 140DAC were consistent with the study results of 2022, the Burley tobacco aged faster under YR 70:30 light. However, at the 170DAC period, YR 70:30 light inhibited the growth of genetic resources significantly compared to the control but caused the ageing of genetic resources faster than the W. This was the initial result of research on lights in the *in vitro*explants, so it was necessary to continue research to determine the appropriate light ratio for genetic resources, ensuring growth inhibition and prolongation storage time, but did not cause ageing of genetic resources during storage.

3.3. Discussion

Light quality affected the number of leaves, plant height and length, and ageing speed of each gene source, and the response of each gene source to light was also different. Research results showed that under Y light, the number of leaves of NC 628 grew the strongest > followed by YB 70:30 and RB 70:30 > YR 70:30, R and W > YB 50:50, RY 50:50 > and lowest was light of B; For Burley 64, R and Y light had the highest number of leaves > then light of RB 70:30, YB 50:50 and RY 50:50 > W, YR 70:30 and YB 70:30 > and lowest was B. Light of R the strongest stimulated to the development of leaves per plant with Burley, while in NC628 genetic resource, the number of leaves of R was equivalent to W. Y light strongly increased the number of leaves in both genetic sources, on the contrary, B light inhibited the growth and development of plant. Compared to other research results, according to L.Y. YANG et al., 2027, R and B light effectively increased the plant growth, while Y light inhibited the growth of *Nicotiana tabacum* L., this result was similar to Su et al. (2014), light of Y and G effectively inhibited the plant growth. According to a study by M.A. Ramírez-Mosqueda L.G et al., 2017, the light of B stimulated increased shoot length, number of roots formed and number of leaves/buds of *Vanilla planifolia*, this result was contrary to our research on 2 tobacco genetic sources.

Different light qualities effectively inhibited differently the tobacco growth compared to light white (L.Y. YANG et al., 2027), similar to research on pepper, lettuce, spinach, and radish (Kim, 2004). B light effectively inhibited the number of leaves of NC628 and Burley 64 genetic sources; YR 70:30 and YB 70:30 light had the same number of leaves as W; and other lights had more leaves than W. For plant height, light of YR 70:30, YB 70:30, YB 50:50, RY 50:50 and B effectively inhibited the plant height compared to W, meanwhile, R and Y light stimulated the growth of plant height compared to W at 110 DAC for the Burley 64. With the NC64 gene source, B effectively inhibited the number of leaves/plant compared to W; YR 70:30 and R had the same number of leaves as W; and other lights had more leaves than W. Light of YR 70:30 and B inhibited maximum plant height, light of YB 50:50 was equivalent to W, and other lights had greater plant height than W. R light increased the plant height of NC628 and Burley 64, and was similar to studies on chrysanthemum, pepper, and *Phalaenopsis* (Schuerger et al., 1997, Kim et al., 2004, Shin et al., 2008). According to L.Y. YANG et al., 2027, light of R reduced the tobacco plant height compared to W (M.A. Ramírez-Mosqueda L.G et al., 2017), and different growth stages affected light types differently. Y and W light increased the plant height the most, and monochromatic light inhibited the plant growth, especially plant height, such as *chrysanthemum*, pepper, and *Phalaenopsis* (Schuerger et al. 1997, Kim et al. 2004, Shin et al. 2008), and also increased the tobacco stem length (Yang, L.Y et al., 2017). Red LED light had a significantly negative effect on shoot length (reduction by 69%) when the duration of light exposure was less than 20 days (Yuanchun Ma et al., 2021). The shoot length of petunia (Fukuda et al., 2011) and rice (Tran and Jung, 2017) was reduced significantly under red LED light when exposed for less than 20 days. However, when the time was increased to more than 20 days, red LED light had no significant effect on shoot length of pepper (Yoonah et al., 2013). Similarly, when the blue LED light treatment was less than 20 days, shoot length was reduced significantly.

Light quality affected root development: According to research results, the largest root length belonged to the light W>then Y and R> YB 70:30 and RB 70:30> YR 70:30> and the lowest was B. According to research by Ramírez-Mosqueda L.G et al., 2017 on *Vanilla planifolia* genetic resources, light B stimulated the development of the largest root length > followed by B: R and R> the lowest was W. Root length did not differ significantly between light qualities. The fresh/dry weight of RB 70:30 was the highest (11.95/0.5g)> YB 70:30 and Y> W, YR 70:30 and R> and the lowest was B (3.79/0.2g). Compare the research results of M.A. Ramírez-Mosqueda et al., 2017 on *Doritaenopsis* tissue culture plants, B light had the highest fresh weight and lowest was the light of W, R and B: R. Dry weight of B light was the highest> then R> W and B: R. Weight Fresh/dried of R was significantly higher than the control (Rita Cassia N. Pedroso et al., 2017). Yang, L.Y et al., 2017 and Ke et al., 2011, the fresh/dry weight of W was larger than R and B.

Light of Y had the highest leaf length > then YR 70:30, YB 70:30, and R > RB 70:30 and W > and the shortest was light of B. For leaf width, YR 70:30 had the largest leaf width > next to Y > YB 70:30 > W and RB 70:30 > lowest was B. Research results were also different from Yang, L.Y et al., 2017, and Ke et al., 2011: Light of Y significantly reduced leaf length and width compared to W, and R light stimulated an increase in leaf length. For B light, the two research results were similar. B light had a strong inhibitory effect on leaf length. According to research by Yang, L.Y et al., 2017, W light had the largest width (23cm) > then B > the lowest was Y light, with a reduction compared to W by 31% and 44%, respectively. Compared to the research on NC628 and Burley 64, light of YR 70:30 and Y effectively increased the leaf width, on the contrary, B inhibited the leaf width development. This difference might occur when the research objects were from different genetic sources because each different genetic source had a different sensitivity level to lights. According to research by Yang, L.Y et al., 2017, the rate of leaf length/width under Y was the highest and then R, compared to research on NC628, under R light, the plant had a leaf length/width ratio highest (1.99) > followed by YB 70:30 > W and Y (1.75 - 1.77) > and lowest was YR 70:30 and B (1.61 - 1.65).

Light quality mainly affected the photosynthetic performance of plants. In the experimental lights, Y light had the highest photosynthetic efficiency > RB 70:30 and R > the lowest was B and YR 70:30. Light of Y, R, and RB 70:30 had higher photosynthetic efficiency than W, other lights have lower PE than W. According to research by Yang, L.Y et al., 2017, the quality of monochromatic light was different in inhibiting plant growth by reducing the activity of the photosynthetic apparatus in plants. R and B light was more effective in promoting photosynthesis, while Y inhibited photosynthesis and plant growth. The rate of photosynthesis increased when plants were exposed to W, R or mixed B and R light (JeongWookHeo et al., 2006). Photosynthetic capacity was highest in leaves treated with blue light, followed by white, red, and green. Adaptation to blue light could improve light use efficiency and reduce photoinhibition when exposed to high levels of sunlight, which could benefit for the plant development (Kang et al., 2021). The mixture of R and B light in a 7:3 proportion that was recommended for gerbera micropropagation positively influenced the functioning of the photosynthetic apparatus. R light depleted the performance of the photosynthetic apparatus and caused its permanent damage. The RB LED spectrum ensured the most efficient non-photochemical quenching of the photosystem II (PS II) excitation state and high shoot quality (Cioć Met al., 2021).

3.4. Conclusion and suggestions

Research results determined the YR 70:30 light that was effective in inhibiting the growth of NC 628, and the plant quality was equivalent to W. For Burley 64, experimental lights were unsuitable for storing Burley's genetic resources. Those lights effectively limited the growth of Burley but caused the ageing quickly of Burley.

Light of YR 70:30 had the effect of inhibiting the growth of 05 genetic resources, and ageing speed was equivalent to W at the 100 DAC period. At the 170 DAC period, YR 70:30 light inhibited the growth and had significantly compared to the W, but caused the ageing of genetic resources faster than white light. The burley genetic resource was more sensitive to YR 70:30 light than flue-cured tobacco.

The light of Y and R effectively increased the leaf number and plant height, and B light sharply limited the growth of explants. Plant dry weight of RB 70:30 and Y was highest, ranging from 0.47 - 0.5g and lowest was B light (0.2g). Under Y, leaf size was larger than other light, reaching 1.9x1.99 cm, and the smallest leaf size belonged to B (2.85x1.73cm).

Next time, we will continue to research mixed light ratio to determine the suitable light for genetic resources, ensuring growth inhibition and prolongation storage time but not causing ageing of genetic resources during storage.

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