

Physiologic Regulation of Mixtalol in Rape Senescence and Its Yield Effects

W. Zhou,^{1,*} S. Tao,¹ and D. Zhao²

¹Department of Agronomy, Zhejiang Agricultural University, Hangzhou 310029, China; ²Central Laboratory, Zhejiang Agricultural University, Hangzhou 310029, China

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Abstract. Physiologic and yield effects of mixtalol at various concentrations sprayed on rape at the anthesis stage were examined. Foliar sprays of 4 and 2 ppm mixtalol significantly increased the chlorophyll content of rape leaves and pods, reduced the accumulation of malondialdehyde and ethylene production, and delayed the degradation of superoxide dismutase and catalase activities of the rape plant. Mixtalol also increased root oxidizability. Meanwhile, the number of branches and pods per plant was increased, and a 10.7% and 8.2% increase of seed yield over the controls was observed with treatments of 4 and 2 ppm mixtalol, respectively. No significant effects from mixtalol were observed on the maturation of plants or on the seed oil content or the erucic acid and glucosinolate content. Total rape oil production increased with 4 and 2 ppm mixtalol significantly by 12.4% and 10.5%, respectively, over the controls.

Plant growth regulators have emerged as popular chemicals that may increase agricultural production at an unprecedented rate and help in removing or circumventing many of the barriers imposed by genetics and the environment (Nickell 1982). Mixtalol (MTL, a mixture of long chain aliphatic alcohols varying in chain length from C₂₄ to C₃₄) is a recent chemical introduction that may stimulate plant growth. It has been reported to have better growth promoting effects and is more stable than triacontanol (Duan et al. 1991, Malik et al. 1987, Srivastava and Menon 1987). This chemical has promoted growth in several crops such as rice, wheat, rape,

and legumes. It has been reported to increase the chlorophyll content and the rate of photosynthesis, enhance yield and yield components, and improve product quality (Malik et al. 1987, Setia et al. 1989, Zhou et al. 1991, 1992). The objectives of the present experiments were to examine further the physiologic effect of MTL with respect to rape senescence and its yield effect under field conditions.

Materials and Methods

Oilseed rape (*Brassica napus*) cv. 601 was used in the experiment. A 2,000-ppm suspension of MTL (a mixture of tetracosanol, hexacosanol, octacosanol, triacontanol, doctriacontanol and tetratriacontanol in varying concentrations) was formulated and provided by the Seed Regulator Laboratory of Zhejiang Agricultural University. The experiments were conducted at the university farm during 1992–1993 and were based on the previous results of MTL for 1990–1991 (Zhou et al. 1992). Four concentrations of MTL were made as follows: 0 (distilled water, control), 2, 4, and 6 ppm. All treatments were applied as foliar sprays at anthesis at the rate of 750 L of diluted solution/ha. Seedlings were transplanted at the five-leaf stage into the experimental plots in a randomized block design using three replicates. Each bed was 4.0 m long and 1.7 m wide, with a total of 80 plants raised. Conventional cultivation methods were followed.

Top leaves 8 and 9 of 10 random plants from central rows of each plot were used for physiologic analyses at 10-day intervals after MTL spraying. Leaf and pod chlorophyll contents were determined by the method of Chen (1984). Leaf malondialdehyde (MDA) and ethylene content were measured using thiobarbituric acid (TBA) and gas chromatography (Dong et al. 1983). Leaf superoxide dismutase (SOD) activity was determined by the method of Zhu et al. (1990), and leaf catalase (CAT) activity was analyzed by the hydrogen peroxide reduction method (Shandong Agricultural College 1980). Root oxidizability was measured by the red tetrazolium (TTC) reduction method (Shandong Agricultural College 1980). Data on plant height, stem width, branching position, number of primary and secondary branches, pods per plant, seeds per pod, and 1,000-seed weight were recorded from 10 random plants at harvest. Harvested seeds from control and MTL-treated plants were analyzed for oil content, erucic acid, and glucosinolates (Chinese Academy of Agricultural Science 1990).

Abbreviations: MTL, mixtalol; MDA, malondialdehyde; TBA, thiobarbituric acid; SOD, superoxide dismutase; CAT, catalase; TTC, tetrazolium.

* Author for correspondence.

Table 1. Effect of MTL on chlorophyll contents (mg/gFW) of rape leaves and pods.

MTL concentration (ppm)	Leaf				Pod	
	10 ^a	20	30	40	30	40
0	1.47	1.22	1.02	0.83	0.378	0.276
2	1.51	1.29	1.06	0.96*	0.403*	0.281
4	1.57*	1.36*	1.10*	0.97**	0.401	0.294*
6	1.48	1.35*	1.00	0.84	0.382	0.275

^a Days after MTL spraying.

* $p < 0.05$; ** $p < 0.01$.

Results

Chlorophyll Content

The chlorophyll contents of leaves and pods were increased after spraying with MTL (Table 1). The highest chlorophyll values were obtained using 4 ppm MTL and gave readings of 6.8, 11.5, 7.8, and 16.9% greater than the controls at 10, 20, 30, and 40 days after MTL spraying for leaves and 6.1 and 6.5% greater than the controls at 30 and 40 days for pods, respectively. It was suggested that the degradation of chlorophyll was retarded, and the MTL-treated plants maintained a higher photosynthetic rate at the late growth stage.

MDA Content and Ethylene Production

The leaf MDA content and ethylene production were gradually increased in the course of rape senescence, and foliar spray of MTL significantly slowed down the MDA accumulation and ethylene production (Table 2). The best effect was achieved by treatments with 4 and 2 ppm MTL, and the MDA content was decreased by 14.8, 12.3, 15.3, and 22.4%, and 7.8, 11.6, 5.9, and 17.1%, respectively, over the controls at 10, 20, 30, and 40 days after MTL spraying, and ethylene production was reduced by 15.3, 22.6, 20.6, and 24.8%, and 2.9, 17.0, 15.9, and 18.6%, respectively, over the controls. The results indicated that MTL could delay the production of ethylene, a promoter of senescence, and lipid peroxidation in rape leaves.

SOD and CAT Activities

MTL also increased the activities of SOD and CAT of rape leaves (Table 3). The highest SOD activities were obtained from 4 ppm MTL treatment and gave readings of 8.0, 7.8, 9.2, and 10.0% greater than the control at 10, 20, 30, and 40 days after MTL spraying; likewise, its CAT activities increased by 5.1,

7.6, 6.9, and 10.7%, respectively, over the control. The SOD and CAT activities were also increased with 2 and 6 ppm MTL, suggesting that MTL could delay the degradation of SOD and CAT activities and prevent damage of cell membranes by superoxide free radicals.

Root Oxidizability

The root oxidizability of rape plants was increased with MTL (Table 4). The 4-ppm MTL-treated plants had the highest root oxidizability, which was significantly increased by 6.8, 12.8, 12.5, and 8.0%, respectively, over the control at 10, 20, 30, and 40 days with MTL spraying. The results indicated that MTL could promote root oxidizability, delay root senescence, and therefore improve root growth.

Yield Components and Seed Yields

There were certain effects of MTL on the morphologic characteristics of rape plants (Table 5). Plant height was insignificantly increased when sprayed with MTL; no obvious differences in stem width and branching position were found between various treatments and the control. The primary branches in 4-ppm MTL-treated plants and the secondary branches in 6-ppm-treated plants were increased by 8.0 and 6.8%, respectively, over the controls.

Table 5 also shows enhancement in pods per plant after MTL treatment. The best effect was obtained from 4 and 2-ppm MTL treatments, and the pods per plant were increased by 9.1 and 7.5%, respectively, over the controls. No significant difference in seeds per pod and seed weight was observed between treatments and controls. The seed yield of 4- and 2-ppm MTL treatments reached 2074.5 and 2028.0 kg/ha, respectively, a significant increase over the controls of 10.7 and 8.2%.

Seed Quality

The oil content and erucic acid and glucosinolate content of rapeseeds are important quality characters. Table 6 indicates that there was only an insignificant increase (0.6–2.1%) in the seed oil content from plants treated with MTL, but the oil yields of 4- and 2-ppm MTL treatments increased significantly by 12.4 and 10.5%, respectively, over the controls. No obvious difference of erucic acid and glucosinolate content of seeds was observed between the treatments and the control. In addition, the maturation of plants was not affected much by MTL. All plants were harvested on the same day.

Table 2. Effect of MTL on MDA and ethylene production of rape leaves.

MTL concentration (ppm)	MDA ($\mu\text{mol/gFW}$)				Ethylene ($\text{nl/gFW} \cdot \text{h}$)			
	10 ^a	20	30	40	10	20	30	40
0	17.42	18.39	20.06	28.84	0.275	0.501	0.734	1.078
2	16.06*	16.26*	18.88	23.92**	0.267	0.416*	0.617*	0.878**
4	14.84*	16.13*	16.99*	22.39**	0.233*	0.388**	0.583**	0.811**
6	17.29	17.03*	20.02	28.77	0.242*	0.450*	0.694	1.067

^a Days after MTL spraying.* $p < 0.05$; ** $p < 0.01$.**Table 3.** Effect of MTL on SOD and CAT activities of rape leaves.

MTL concentration (ppm)	SOD activity ($\text{unit/gFW} \cdot \text{min}$)				CAT activity ($\text{H}_2\text{O}_2 \text{ mg/gFW} \cdot \text{min}$)			
	10 ^a	20	30	40	10	20	30	40
0	38.06	30.87	18.44	16.26	20.72	19.88	19.58	18.62
2	41.11*	30.90	19.41	17.21	21.38	21.16*	19.76	19.29
4	41.10*	33.29*	20.14*	17.88*	21.78	21.39*	20.93*	20.61*
6	38.70	31.59	18.16	16.35	21.50	21.07	19.71	19.41

^a Days after MTL spraying.* $p < 0.05$.**Table 4.** Effects of MTL on root oxidizability (TTC $\text{mg/g FW} \cdot \text{h}$) of rape plants.

MTL concentration (ppm)	10 ^a	20	30	40
0	44.00	33.25	28.05	25.05
2	44.75	36.50*	29.50	28.50*
4	47.00*	37.50*	31.55*	27.05*
6	43.50	37.25*	30.02*	24.00

^a Days after MTL spraying.* $p < 0.05$.

Discussion

The 1- to 3-week period after anthesis is considered very critical in determining yield capacity, pod number, and seed number in rape plants (Chinese Academy of Agricultural Science 1990). More than 75% of the pods that attain maturity are formed from flowers that open within 2 weeks of anthesis, and a restricted supply of carbon assimilates during the time of anthesis reduces the pod number (Tayo and Morgan 1979). Thus, MTL was sprayed on rape at the anthesis stage, and it was found that it significantly increased the chlorophyll content of rape leaves and pods, as reported in our previous experiments and in other crops such as rice, wheat, and legumes (Malik et al. 1987, Zhou and Xi 1993). MTL also prolonged leaf longevity, increased green pod area, enhanced carboxylation and photophosphory-

lation activities, and therefore increased the photosynthetic rates of the plant (Zhou et al. 1992, Malik et al. 1987). In addition, MTL had been reported to control stomatal movement and suppress the rate of photorespiration and was one of the few chemicals that was effective for both the processes of photosynthesis and respiration in an advantageous manner (Menon and Srivastava 1984).

Plant senescence is associated with the degradation of chlorophyll, the accumulation of MDA and ethylene production, the decrease of SOD and CAT activities, and the root oxidizability (Dong et al. 1983, Lin et al. 1984). MDA is a product of lipid peroxidation and causes damage to enzymes and plant membranes (Halliwell 1981). Ethylene can accelerate plant senescence and leaf abscission. SOD, together with CAT and peroxidase, can prevent damage to cell membranes by superoxide free radicals (Zhu et al. 1990). The present experiment indicated that the foliar sprays of MTL could delay the decrease of SOD and CAT activities and chlorophyll content, reduce the accumulation of MDA and ethylene production, and promote root oxidizability. Therefore, the physiologic function of cells was improved to some extent, and plant senescence was delayed by MTL.

The physiologic and yield effects of MTL foliar sprays at anthesis varied with concentrations, and better effects were obtained from 4- and 2-ppm MTL treatments than from 6-ppm treatment. It was recalled that the effective concentration of triacon-

Table 5. Effect of MTL on yield components and yield of rape.

MTL concentration (ppm)	Plant height (cm)	Stem width (cm)	Branching position (cm)	Primary branch (No.)	Second branch (No.)	Pod/plant (No.)	Seed/pod (No.)	1,000 seed weight (g)	Seed yield (kg/ha)
0	154.7	1.83	32.0	7.5	5.9	359.1	17.2	3.62	1873.5
2	158.8	1.90	30.8	7.6	6.0	386.2*	17.7	3.74	2028.0*
4	161.5	1.88	31.3	8.1*	5.8	391.9*	18.0	3.77	2074.5*
6	154.4	1.90	29.9	7.8	6.3*	370.6	17.7	3.65	1945.5

* $p < 0.05$.**Table 6.** Effect of MTL on rapeseed quality.

MTL concentration (ppm)	Oil content (%)	Oil yield (kg/ha)	Erucic acid (%)	Glucosinolate ($\mu\text{mol/g}$)
0	39.02	657.9	49.40	118.0
2	39.85	727.3*	49.08	117.6
4	39.60	739.4*	49.37	115.1
6	39.25	687.2	49.21	118.3

* $p < 0.05$.

tanol, once widely applied in the field crops, ranged from 0.5 to 1.0 ppm (sprayed twice at weekly intervals), and higher concentrations inhibited plant growth (Shen 1990). At our previous similar experiment, 4- and 2-ppm MTL-treated plants showed significant photosynthetic and yield effects, whereas 6- and 8-ppm MTL treatments revealed insignificant photosynthetic effect or yield reduction (Zhou et al. 1992). Therefore, the suitable concentrations for MTL foliar sprays at rape anthesis ranged from 4 to 2 ppm, which had the best physiologic and yield effects and no adverse effect on seed quality.

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References

- Chen FM (1984) Determining the chlorophyll contents of plant leaves by acetone/ethanol mixture assay. *For Sci Commun* 2:4–8
- Chinese Academy of Agricultural Science (1990) Rape cultivation of China. Agriculture Press, Beijing, pp 173–175
- Chinese Academy of Agricultural Science (1990) Rape cultivation of China. Agriculture Press, Beijing, pp 536–545
- Dong JG, Yu ZW, Yu SW (1983) Effect of increased ethylene production during different periods on the resistance of wheat plants to waterlogging. *Acta Phytophysiol Sin* 9: 383–389
- Duan XM, Yu YL, Fan LJ (1991) An introduction to the physiological function and application of mixtalol. *New plant growth regulators (special ed)*. Plant Growth Substances Association of China, Hangzhou, pp 79–81
- Halliwell B (1981) Chloroplast metabolism: The structure and function of chloroplasts in green leaf cells. Clarendon Press, Oxford, p 186
- Lin ZF, Li SS, Lin GZ, Sun GC, Guo JY (1984) Superoxide dismutase activity and lipid peroxidation in relation to senescence of rice leaves. *Acta Bot Sin* 26:605–615
- Malik CP, Setia RC, Setia N (1987) Enhancing crop yield and improving quality of produce by long chain alcohols. In: Srivastava HC, Bhaskaran S, Menon KKG (eds) *Crop productivity*. Oxford, New Delhi, pp 117–125
- Menon KKG, Srivastava HC (1984) Increasing plant productivity through improved photosynthesis. *Proc Ind Acad Sci* 93:359–370
- Nickell LG (1982) *Plant growth regulators: Agricultural uses*. Springer-Verlag, Berlin
- Setia RC, Richa, Setia N, Ahuja KL, Malik CP (1989) Effect of mixtalol on growth, yield and yield components of Indian mustard (*Brassica juncea*). *Plant Growth Regul* 8:185–192
- Shandong Agricultural College (1980) A guide to plant physiology experiment. Shandong Science and Technology Press, Jinan, pp. 109–190
- Shen YQ (1990) *Plant growth regulators*. Chemical Industry Press, Beijing, pp 219–220
- Srivastava HC, Menon KKG (1987) Mixtalol: A new growth regulator for increasing crop productivity. In: Srivastava, HC, Bhaskaran S, Menon KKG (eds) *Crop productivity*. Oxford New Delhi, pp 43–55
- Tayo TO, Morgan DC (1979) Factors influencing flower and pod development in oilseed rape (*Brassica napus*). *J Agric Sci* 92:363–373
- Zhou WJ, Xi HF (1993) Effects of mixtalol and paclobutrazol on photosynthesis and yield of rape (*Brassica napus*). *J Plant Growth Regul* 12:157–161
- Zhou WJ, Shen HC, Duan XM, Ye JY (1991) The yield effect of mixtalol on *Brassica napus*. *Acta Agric Univ Zhejiang* 17:70
- Zhou WJ, Xi HF, Li X (1992) Effects of mixtalol on the photosynthesis and yield of *Brassica napus*. *Acta Agric Univ Zhejiang* 18:23–27
- Zhu GR, Zhong HW, Zhang AQ (1990) *Plant physiology experiment*. Peking University Press, Beijing, pp 242–245