Agricultural Journal 7 (3): 177-179, 2012
ISSN: 1816-9155

Effects on Polyploidy Induction in Asparagus Lettuce of Concentration and Presoaking Time of Colchicine

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Abstract: Different concentration and presoaking time of colchicine to induce polyploidy in asparagus lettuce were studied in this research. The results showed that the colchicine had an obvious inhibiting effect on asparagus lettuce sprout and the inhibitory effect is more obvious with increasing presoaking time of colchicine. Asparagus lettuce plant through colchicine presoaking can increase the diameter of stems, reduce the plant height and increase stoma density. The sprout ratio and variant plants treated with 100 mg L^{-1} colchicine concentration 32 and 48 h, 500 mg L^{-1} colchicine concentration 4, 8 and 48 h and 1000 mg L^{-1} colchicine concentration 4 h were better and can be used in production.

Key words: Colchicine, concentration, presoaking time, polyploidy, asparagus lettuce

INTRODUCTION

Polyploidy is one of the most common phenomena in nature. Sun and Zhang (2004) had showed that some 65% of monocotyledons and 40% of dicotyledons are polyploid as are 90% of ferns, many bryophytes and some algae. Qiao and Song (2002) indicated that polyploids generally thaw some good characters for example, vigorous plant, higher biological yield, bigger seed, good quality and special resistantance.

Quan *et al.* (2004) obtained the homologous tetraploid of Arctium lappa plants from seed treatment by colchicine, also got some octoploids and aneuploid plants at the same time. Mo *et al.* (2010) have showed that method for polyploidy induction in dendranthema lavandulifolium was soaking seeds in 500 mg L^{-1} colchicum for about 12 h and the induction ratio was about 23%. The polyploid is very important in vegetable breeding and has a wide prospect for development and utilization.

Asparagus lettuce (*Lactuca sativa* L.var. *asparagina Bailey*) is annual or biennial vegetables. Lettuce with rich nutrients is broadly cultivated around the whole years in both North and South of China. The diameter of stems and its fresh and tender have great effects on quality and quantity in asparagus lettuce.

However, conventional breeding methods to improve the diameter of stems and its fresh and tender was mainly depending on plant growth hormon and fertilizers. Little study on genetic improvement, Zhang *et al.* (1997) inducted polyploidy in asparagus lettuce with colchicine by tissue culture and emphasis on the treatment processes. In this experiment, different concentration and presoaking time of colchicine to induce polyploidy in asparagus lettuce were studied to obtained the optimal conditions for inducing polyploidy and create a new inducing way.

MATERIALS AND METHODS

Asparagus lettuce seeds were supplied by Sichuan Mianyang Kexing Seeds Co., Ltd. and the variety is round leaves asparagus lettuce. Colchicine were supplied by Shanghai Chemical Reagent Factory and its content was 97%.

Experimental design and statistical analysis: The experiment was conducted with 3 replications in double factors randomized block design and 30 seeds uniform per replication. Colchicine concentration: 100, 500, 1000, 2000 and 4000 mg L^{-1}. Presoaking time: 4, 8, 16, 32 and 48 h. When deal with the seeds, firstly, clean it with water, after air dries place them in different concentration and record the presoaking time.

Secondly, clean the seeds after soaking again then place seeds in a constant temperature seed accelerating germination device with 20°C to accelerate germination. Thirdly, 10 days after accelerating germination, the sprouts rate would be recorded. The germinating seeds were sowed in farm directly on December 10, 2010 then

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seedling quantity in field were recorded on January 20, 2011. The plants were planted on February 15, 2011. The stoma number, width of blade, plant height and stem diameter were observed and measured on April 12, 2010. Fertilizer and water management, insect pest and disease controls and other field management technique were the same as common ways of cultivation and management.

RESULTS AND DISCUSSION

Effects on germination rate of colchicine in asparagus lettuce seeds: Figure 1 shows that the germination rate reduced with the presoaking time of colchicine increased at the same concentration after accelerating germination. Especially, the germination rate was 75% with the 4000 mg L^{-1} and presoaking 48 h.

The germination rate changed very little at the same presoaking time but different concentration which can be showed that presoaking time played an important role in germination and the more obvious inhibition, the more presoaking time at the same concentration of colchicine.

Effects on planting quantity of colchicine in asparagus lettuce seeds: From Table 1, the planting quantiy 40 days after sowing were recorded which indicated that colchicine had obvious inhibited planting with the increased concentration of colchicine and presoaking time. The planting number was zero when the concentration of colchicine was >1000 mg L^{-1} so, there is not much point in doubling the asparagus lettuce polyploidy with high concentration colchicine and long presoaking seeds.

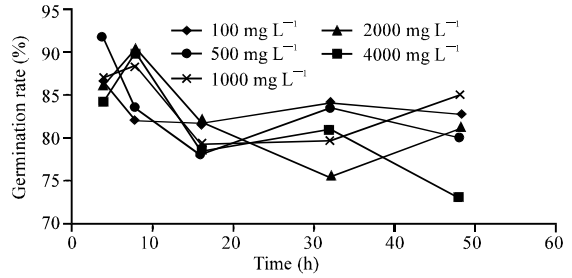

Fig. 1: Effects on germination rate of colchicine in asparagus lettuce seeds

Table 1: Effects on planting quantity of colchicine in asparagus lettuce seeds

	Planting No. (h)				
Concentration (mg L^{-1})	4	8	16	32	48
100	6.7^{a}	3.0^{b}	3.3^{b}	3.3^{b}	1.3^{d}
500	2.0^{cd}	1.3^{d}	2.7^{bc}	0.0^{e}	0.0^{e}
1000	1.3^{d}	0.0^{e}	0.0^{e}	0.0^{e}	0.0^{e}
2000	0.0^{e}	0.0^{e}	0.0^{e}	0.0^{e}	0.0^{e}
4000	0.0^{e}	0.0^{e}	1.0^{de}	0.0^{e}	0.0^{e}

Effects on plant trait of colchicine in asparagus lettuce plant: Table 2 shows that the colchicine had obvious inhibited plant height with the increased concentration in some concentration range. There were no more changes on plant height when concentration are >1000 mg L^{-1} and there were certain influence on plant height at the same low concentration but different presoaking time. When the concentration was 100 mg L^{-1} among 5 treatments of presoaking time, only the treatment of 48 h were significantly different with the treatment of 4 and 8 h and the difference of other treatments was not significantly. There were no significant difference on plant height when concentration was >500 mg L^{-1}.

The colchicine had obvious increased stem diameter with the increased concentration and presoaking time but the concentration was a more important influence than presoaking time. There was no significant difference in stem diameter when concentration was >500 mg L^{-1} and presoaking was >8 h.

The increase of concentration colchicine did not significantly affect the width of blade. The treatment with concentration 100 mg L^{-1}, presoaking 16 h was significantly different with the treatment of concentration 500 mg L^{-1}, presoaking 8 h and concentration 100 mg L^{-1}, presoaking 4 h and the difference of other treatments was not significant.

Effects on stoma number of colchicine in asparagus lettuce plant: Table 3 shows that with the increased concentration and presoaking time, the stoma number had reduced. It had the most stoma number when the concentration 100 mg L^{-1}, presoaking time 4 or 8 h which had a significant difference with the treatment presoaking time 32 and 48 h and treatments 500 cm L^{-1}, 1000 and 4000 mg L^{-1}. We can also seen that there was no significant difference between presoaking 8 and 16 h when the concentration was 500 mg L^{-1} and the stoma number was seven.

Table 2: Effects on plant height,stem diameter and width of blade of colchicine in asparagus lettuce

Treatments				
Concentration (mg L^{-1})	Presoaking time (h)	Plant height (cm)	Stem diameter (cm)	Width of blade (cm)
100	4	63.2^{a}	3.000^{e}	7.2^{bc}
100	8	60.3^{3a}	3.200^{de}	7.9^{abc}
100	16	57.2^{ab}	3.300^{cde}	8.4^{a}
100	32	55.6^{ab}	3.440^{cd}	8.1^{ab}
100	48	51.2^{bc}	3.920^{ab}	8.0^{ab}
500	4	45.7^{cd}	3.600^{bc}	7.3^{abc}
500	8	46.0^{cd}	3.900^{ab}	6.8^{c}
500	16	42.4^{d}	3.900^{ab}	7.5^{abc}
1000	4	33.7^{e}	3.900^{ab}	7.3^{abc}
4000	16	33.5^{e}	4.200^{a}	7.4^{abc}

Table 3: Effects on stoma number of colchicine in asparagus lettuce

Treatments		
Concentration (mg L^{-1})	Presoaking time (h)	Stoma No.
100	4	12.0[a]
100	8	12.0[a]
100	16	11.7[a]
100	48	10.0[b]
100	32	9.7[b]
500	4	9.0[b]
500	8	7.7[c]
500	16	7.0[c]
1000	4	5.0[d]
4000	16	4.3[d]

CONCLUSION

It was only preliminary research to use the plant traits to identify the polyploid in the study. However, most newly arisen polyploids fail to become established because of meiotic abnormalities and the paucity of appropriate mates it need observe the chromosome to determine the doubling of the chromosome number. The stoma number and other plant traits can serve as a reference for the identification of polyploidy but Chimera and tetraploid should be observed by microscopic observation. Ji *et al.* (2011) had showed that the treated shoots of Chybridium with 0.05% colchicine for 24 h had a higher inducing rate which was up to 28.2%. Shen *et al.* (2011) indicated that the mutation rate of seeds was 53.3% with the treatment of 0.3% colchicines for 48 h. Liu *et al.* (2011) had showed that best concentration of colchcine was 0.1% in chromosome doubling induction of *L. davidii* var. unicolor (Hoog) cotton.

The sprout ratio and variant plants treated with 100 mg L^{-1} colchicine concentration 32 and 48 h, 500 mg L^{-1} colchicine concentration 4 , 8 and 48 h and 1000 mg L^{-1} colchicine concentration 4h were better and can be used in production.

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