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Selections and characters of L-Hydroxyproline resistant *Medicago sativa* L. varieties

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Key words: *Medicago sativa* L. callus, hydroxyproline-resistant, physiological and biochemical indexes

Introduction As animal husbandry develops, more researchers are concerned about the quality and the adaptability of pasture forages in the different environment conditions. Alfalfa (*Medicago sativa* L.) as the king of forages plays an important role in improving ecological environment and increasing quantity of legumes with high quality. The stress resistant study of alfalfa has always been emphasized.

Materials and methods Callus of alfalfa Aohan and Longmu 801 were used in this study.

Semilethal experiment of L-Hyp: The alfalfa callus was divided into 0.5cm squares and then implanted into the MS culture medium with the content of 0, 4, 8, 10, 16mmol L-Hyp/L-respectively. The callus was cultured for 25 days, and the survival ratio was calculated to determine the lethal dose and the semilethal dose.

Mutations of callus: The alfalfa callus was divided into 0.5cm squares and mutated by the liquid culture medium containing NaN₃ for 1 hour. The mutant callus was cultured in MS culture medium containing 8mmol L-Hyp/L-at 25°C for 30 days. The survival callus was then implanted into MS culture medium and cultured for 20 days. At last, the callus was cultured in MS culture medium containing 8mmol L-Hyp/L.

Psychological index: The content of free proline was analyzed by sulfosalicylic acid dehydrate-acidic ninhydrin method; the content of soluble sugar and the activity of POD were analyzed by the method of Zhang Zhiliang.

Resistance of mutant cells to the stress of PEG and low-temperature: The mutant callus of Aohan and controls were divided into 0.5cm squares and separately cultured in the lipid culture medium containing 0, 100, 200, 300 and 400g/L PEG for 24h. The callus was then implanted into the mutant culture medium to calculate the survival ratio after 25d. The mutant callus of Longmu 801 and controls were divided into 0.5cm squares and respectively cultured in the culture medium at 8°C, 4°C, 0°C, -4°C, -8°C for 10d. The callus was then cultured normally and then the survival ratio was calculated.

Results and discussion

Sensitivity of callus to L-Hyp: The callus growth of control was restrained intensively as the concentration of L-Hyp increased. The semilethal dose of L-Hyp to callus was 8mmol/L, while the lethal dose was 16mmol/L.

The resistant psychological index: The mutant callus was marked with the high content of proline, which is 2.84 (Aohan) and 3.17 (Longmu 801) times more than that of the control respectively. The content of soluble sugar in mutant callus is 1.56 (Aohan) and 1.76 (longmu 801) times more than that of the control respectively. There was a significant difference in the activity of POD between mutant callus and controls. Results of the resistance of mutant callus to the stress of PEG and low-temperature indicated that the mutant callus have a higher resistant ability and adaptation to the extremely adverse environmental conditions, such as dry or cold.

Conclusions When the NaN₃ is used as mutant agent and the proline analogs (Hyp) is used as the selective stress, the resistance to Hyp of varieties was significantly different between the culture medium containing 8mmol/L Hyp and that without Hyp. Results of psychological index test indicated that the mutant callus have a high stress resistance and could adapt to the adverse environment.

Reference

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