Comparative Study on Low Temperature Germination Ability of Overwintering Green Manure

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Abstract. Green manure is a biological fertilizer source with complete nutrients, which provides reference for the selection of green manure resistant to low temperature germination. Six green manure crops including \textit{Brassica napus} \textit{L.}, \textit{Orychophragmus violaceus} \textit{(L.) O. E. Schulz}, \textit{Medicago sativa} \textit{L.}, \textit{Lolium perenne} \textit{L.}, \textit{Melilotus officinalis} \textit{(Linn.) Pall.}, and \textit{Vicia villosa} \textit{Roth} were selected as experimental materials to study the effects of low temperature stress on germination and seedling growth of different green manure through low temperature germination experiments. The results showed that there were significant differences in cold tolerance of the seeds of 6 medium green manure crops during germination. The germination potential, germination rate, germination index, vitality index, seedling height, root length, seedling fresh weight, soluble protein content and chlorophyll content of \textit{Brassica napus} \textit{L.} and \textit{Vicia villosa} \textit{Roth} seeds were all better than those of other green manure crops, which provided theoretical basis for the actual production of green manure.

Keywords: Green Manure, Low Temperature, Germination Index, Seedling Growth, Physiological and Biochemical Indexes
1. Introduction

Green manure refers to cultivated plants that are mainly used as fertilizers. It is applied by turning over and pressing fresh plants in situ, or retting, stacking and then applying in different places. Green manure is mostly leguminous plants, but also gramineous and cruciferous plants. Due to the continuous improvement of multiple cropping index and unit yield, the release and utilization of soil nutrients are fast but difficult to supplement in time. Planting green manure can quickly return organic matter and minerals to the soil to balance and supplement nutrients. Green manure has the functions of renewing soil humus, increasing soil organic matter, improving soil physical and chemical properties and increasing soil phosphorus availability [1]. Research by Wang Lu et al. [2] shows that returning Astragalus sinicus L. and Olyza Sativa L. to the field can reduce soil bulk density and improve soil porosity. The effect on improving soil nutrients by plowing is more significant. Zhou Kaifang et al. [3] Through the experimental study on the two treatments of green manure turning over and not turning over (control), it was found that compared with the physical and chemical properties of the topsoil before the experiment was carried out, the organic matter, alkali-hydrolyzable nitrogen, available phosphorus and available potassium of the soil could be increased by 0.07%, 1.9 mg/kg, 4.7mg/kg and 3.1 mg/kg respectively after the green manure turning over. The pH value of soil pH changed from 5.5 to 6.0, the bulk density of soil decreased by 0.05g/cm3, and the porosity increased by 1.9%. Most of the nitrogen fixed by the growth of green manure crops can be directly returned to the soil by turning over and returning to the field, thus effectively improving the nitrogen supply capacity of the soil and significantly improving the soil fertility and productivity [4-6]. In recent years, with the national policy of weight loss and drug reduction, the planting area of green manure in the north has gradually expanded in recent years. Different green manure is easy to suffer from low temperature stress before sowing in winter. Previous researches on the effects of winter green manure on soil physical and chemical properties and biological properties are more, and researches on the effects of low temperature stress on the emergence of green manure are relatively few. Therefore, this study studies the effects of different overwintering green manure on emergence and seedling growth under low temperature stress through low temperature experiments, providing theoretical basis for large-scale demonstration and popularization of overwintering green manure in the north.

2. Materials and Methods

2.1. Test Materials

Six kinds of green manure crops including Brassica napus L. Orychophragmus violaceus (L.) O. E. Schulz, Medicago sativa L., Lolium perenne L., Melilotus officinalis (Linn.) Pall. and Vicia villosa Roth were used in the experiment.

2.2. Test Methods

For each treatment, 4 layers of filter paper, 100 seeds, 2 layers of filter paper and a little water are placed in the germinating box. Each green manure is repeated 3 times and put into the germinating box for germinating test. The emergence number was recorded every day and the plant weight was measured on the 7th day.
From the green manure seeds used in the experiment, the seeds with the same grain fullness, size, shape and maturity were respectively selected, sterilized with 1% NaClO for 5 minutes, and then rinsed with distilled water for later use. According to the International Seed Inspection Regulations, the indoor standard germination test of seeds is carried out, then 100 seeds are placed on germination paper, repeated for 3 times, the germination box is placed in an illumination incubator for cultivation, the germination box is treated for 7 days in an incubator at 10 DEG C, and then is transferred to 20 DEG C for further cultivation for 7 days, the number of seeds that normally germinate is counted day by day, the germination number is recorded every day, the germination potential is calculated for 4 days, the germination rate is calculated for 7 days, the seedlings are taken out after 7 days, the fresh weight of the seedlings is weighed, and the germination rate, germination potential and vitality index are calculated. The plant height and root length were measured respectively. The contents of soluble protein and chlorophyll in leaves were measured. The soluble protein content was measured by Coomassie Brilliant Blue G-250 and the chlorophyll content (SPAD) was measured by portable analyzer.

Germination Potential (GE)= Number of Germinated Seeds on Day 4/Number of Seeds Tested ×100%

Germination rate (GR)= number of seeds germinated on the 7th day/number of seeds tested ×100%

Germination index (GI)=\[\sum G_t / D_t\] (Gt is the number of germinating seeds on day t; Dt is the number of germination days in response)

Vitality index (VI)=GI×S (GI is germination index; S is the length (cm) or weight (g) of seedlings within the specified date

3. Results and Analysis

3.1. Effects of Low Temperature Stress on Germination Indexes of Different Overwintering Green Manures

| Varieties                        | GE (%) | GR (%) | GI   | VI   |
|----------------------------------|--------|--------|------|------|
| Brassica napus L                 | 93.33a | 96.00a | 17.12a | 79.69a |
| Orychophragmus violaceus (L.)    | 53.00f | 60.67e | 11.85d | 63.56f |
| Medicago sativa L                | 81.33c | 83.67b | 15.74b | 74.01c |
| Lolium perenne L.                | 73.00d | 73.00c | 14.01c | 72.51d |
| Melilotus officinalis (Linn.) Pall. | 68.00c | 68.00d | 12.47d | 66.31e |
| Vicia villosa Roth               | 84.00b | 84.00b | 16.52ab | 75.87b |

As can be seen from Table 1, the germination potential, germination rate, germination index and vitality index of different green manure under low temperature stress are different. Brassica napus L.
has the highest germination rate and germination potential, which is significantly higher than other green manure seeds. The germination potential and germination rate of Vicia villosa were the second, while the germination potential and germination rate of cymbidium sinense were the lowest, only 53% and 60.67%, which were significantly lower than other treatments. The germination index of 6 green manure varieties ranged from 11.85 to 17.12, with Brassica napus L. having the highest germination index, while the germination index of orchid and Melilotus officinalis (Linn.) Pall. were lower, significantly lower than other treatments. The highest vitality index was Brassica napus L., reaching 79.69, which was significantly higher than other green manure. the sequence of vitality index was Brassica napus L. > Vicia villosa Roth > Medicago sativa L. > Lolium perenne L. > Melilotus officinalis (Linn.) Pall. > Orychophragmus violaceus (L.).

3.2. Effects of Low Temperature Stress on Growth of Different Overwintering Green Manure Seedlings

Table 2 Effects of Low Temperature Stress on Seedling Growth of Different Green Manure Seeds

| Varieties                  | Seedling height (cm) | Root length (cm) | Fresh weight of plant (g) |
|----------------------------|----------------------|------------------|---------------------------|
| Brassica napus L           | 9.44a                | 7.45a            | 15.21a                    |
| Orychophragmus violaceus (L.) | 6.89b               | 6.02bc           | 11.85c                    |
| Medicago sativa L.         | 8.67a                | 6.94ab           | 13.25b                    |
| Lolium perenne L.          | 7.40b                | 6.56abc          | 12.96b                    |
| Melilotus officinalis (Linn.) Pall. | 6.47b              | 5.86c            | 11.47c                    |
| Vicia villosa Roth         | 8.96a                | 7.30a            | 13.67b                    |

Table 2 shows that the seedling height, root length and fresh weight of different green manure seeds are different under low temperature stress. The seedling height of Brassica napus L. is 9.44cm, significantly higher than that of Orychophragmus violaceus (L.), Lolium perenne L. and Melilotus officinalis (Linn.) Pall., and higher than that of Medicago sativa L. and hairy vetch, but the difference is not significant. The root length of Brassica napus L. and Melilotus officinalis (Linn.) Pall. is the longest, of which Brassica napus L. root length is 7.45cm, which is significantly higher than that of Orychophragmus violaceus (L.) and Melilotus officinalis (Linn.) Pall.. The root length is 19.2% and 27.1% higher than that of Orychophragmus violaceus (L.) and Melilotus officinalis (Linn.) Pall. respectively, and there is no significant difference between them and other varieties. The fresh weight of different varieties of green manure is different, with Brassica napus L. reaching up to 15.21cm, which is significantly higher than that of the other five varieties of green manure. The fresh weight of plants of Eryuelan and Melilotus suaveolens is lower, which is 22.1 and 24.6% lower than that of the control.

3.3. Effects of Low Temperature Stress on Physiological Indexes of Leaves of Different Overwintering Green Manure Seedlings

The contents of soluble protein and chlorophyll in leaves of different green manure seedlings showed
differences under low temperature stress. The soluble protein content of *Brassica napus* L. was the highest, significantly higher than that of other treatments. The soluble protein content of *Orychophragmus violaceus* (L.) is the lowest, and the difference among the soluble protein content of six green manure has reached significant level. The chlorophyll content of *Brassica napus* L. is the highest, reaching 49.5, which is significantly higher than that of the other five treatments. The chlorophyll content of *Melilotus officinalis* (Linn.) Pall. is the lowest, which is 39.8% lower than that of *Brassica napus* L.. The chlorophyll content of different varieties is significantly different.

**Table 3** Effects of Low Temperature Stress on Chlorophyll and Soluble Protein Contents in Leaves of Different Green Manure Seedlings

| Varieties                          | Soluble protein content (µg/g FW) | Chlorophyll content (SPAD) |
|-----------------------------------|----------------------------------|---------------------------|
| *Brassica napus* L                | 32.44a                           | 49.5a                     |
| *Orychophragmus violaceus* (L.)   | 22.52f                           | 37.1e                     |
| *Medicago sativa* L.              | 27.58c                           | 40.6d                     |
| *Lolium perenne* L.               | 25.91d                           | 45.3c                     |
| *Melilotus officinalis* (Linn.) Pall. | 24.73e                          | 35.4f                     |
| *Vicia villosa* Roth              | 28.96b                           | 47.3b                     |

### 4. Discussion and Conclusion

The degree of chilling injury during green manure germination mainly depends on low temperature and treatment time. The key to the cold tolerance of green manure is in the germination stage and seedling stage. It is reasonable to comprehensively identify the cold tolerance of plants through the size difference of different indexes under low temperature stress. It is difficult to distinguish the cold tolerance of plants with a single index [7-8]. This experimental study found that some green manure showed strong cold resistance under low temperature stress from a single index, but the cold resistance of this variety was average when all indexes were evaluated during germination. This study found that the germination index, seedling growth index and physiological and biochemical indexes of *Brassica napus* L. and vetch are superior to other green manure, showing strong cold resistance.

The cold tolerance of green manure is controlled by different genes in different periods. With the growth and development of plants, the cold tolerance will change. The cold-resistant varieties screened by the cold-resistant identification in bud stage can only represent that the varieties have strong cold resistance in bud stage and seedling stage. However, the growth and stable yield of green manure depend not only on the two early growth stages of bud and seedling, but also on the formation of reproductive organs when the temperature is too low in the middle and late stages, thus prolonging the later growth stage and reducing the yield. Therefore, the selection of cold-resistant green manure should be based on the analysis of local climate characteristics. The cold-resistance in bud stage is the key to the low-temperature seedling formation of green manure, which should be paid attention to in
the selection process of green manure.

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References

[1] Yunming X, Guoqin H, Shubing W, et al. Effects of Paddy Field Rotation on Soil Physical and Chemical Properties and Crop Yield[J]. Journal of Agricultural Science and Technology, 2004, 6(4): 42–45. (In Chinese)

[2] Lu W, Jianfu W, Xiaohua P, et al. Effects of Astragalus sinicus and straw returning to field and no-tillage and throwing cultivation on rice yield and soil fertility [J]. Chinese Agricultural Science Bulletin, 2010, 26 (20): 299–303. (In Chinese)

[3] Kaifang Z, Yan H. Effects of Leguminosae Winter Green Manure Turning on Soil Fertility and Yield and Quality of Hybrid Maize [J]. Guizhou Agricultural Sciences, 2003, 31:42-43. (In Chinese)

[4] Aulakh M.S, T.S Khera, J.W Doran, et al. Yields and nitrogen dynamics in a rice-wheat system using green manure and inorganic fertilizer[J]. Soil Sci, 2000 64:1867-1876.

[5] Chalk PM. Dynamics of biologically fixed N in legume-cereal rotations: a review[J]. Aust J Agric Res, 1998, 49: 303-316.

[6] Cline G.R, A.F Silvernail. Residual nitrogen and kill date effects on winter cover crop growth and nitrogen content in a vegetable production system[J]. Hort Technology, 2001, 11:219-225.

[7] Hope H J, White P R, Dwyer LM, et al. Low temperature emergence potential of short season corn hybrids grown under controlled environment and plot conditions[J]. Canadian Journal of Plant Science, 1992, 72:83-91.

[8] William G, Hopkins. Introduction to Plant Physiology[M]. New York: John Wiley & Sons, Inc, 1995: 431-432.