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# 大穗黑麦草抗性的有关生理生化指标的研究

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**摘 要**：通过对大穗黑麦草生物学性状观察及测定叶绿素含量，丙二醛含量，抗坏血酸氧化酶活性等生理生化指标，并与湖北省当前推广的小麦品种鄂恩1号、鄂恩4号作比较，结果表明它具有较高的抗性，为小麦的抗性育种提供了新的野生种资源。

**关键词**：大穗黑麦草；生理生化指标；抗性

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# Study on the resistance of physio-biochemical parameter of *Lolium grandispicum* Y. J. Fei

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**Abstract**: In this paper, biological properties of *L. grandispicum* Y. J. Fei were observed and its physio-biochemical criterion such as chlorophyll content, malondialdehyde content and ascorbic acid oxidase activity were measured. After comparing with wheat variety EN1 and EN4 in Hubei, the result suggested that the grass has a good resistance. And the new species can provide new wild species resource for wheat resistant breeding.

**Key words**: *L. grandispicum* Y. J. Fei; physio-biochemical parameter; resistance

*Lolium grandispicum* Y. J. Fei is a type of new species belonging to Hubei *Lolium*. It was first discovered by Fei Yongjun, a teacher of Hubei Agricultural College in the eastern suburb of Jingzhou Hubei in June, 1998(Fei, 1999). The relevant research report has not published because of the short period of discovery.

Wheat is one of the most important cereal crops in the world. However, with the ever-increasing production level and ever-changing environment, there are continuous new requirements on wheat breeding. The genetic resources within wheat species cannot keep up with the breeding. So it is crucial to open new genetic resources to make wheat hereditary basis abundant(Liu, 1993). Whereas *L. grandispicum* Y. J. Fei can be utilized as a sort of new resource of wheat distant hybridization. The paper primarily made a preliminary observation on biological properties of *L. grandispicum* Y. J. Fei and observed the biochemical parameter related to *L. grandispicum* Y. J. Fei and wheat so as to provide theoretical and practical basis for the breeding and improvement of hereditary character of wheat.

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# 1 Material and methods

## 1.1 Material

The experimental materials were *L. grandispicum* Y. J. Fei, wheat variety EN 1 and EN 4. The experimental field was in the whea breeding field of Hubei Agricultural College, China. And the experiment has proceeded from 1999 to 2001.

## 1.2 Methods

(1) Chlorophyll contents were measured by SPAD-502 chlorophyll trochometer produced in Japan. Every material measured 30 plants, and the lamina measured by chlorophyll trochometer was the spreading lamina next to the last. The averaged means were recorded. The measuring part was the center of the lamina.

(2) Malondialdehyde contents were measured by the method of sulfo-barbituric acid (Bai *et al*, 1996): The leaf next to the last was used in the experiment. It was first cleared and dried, then subsections were cut to 0.5 cm in those of 0.3 g in weight were selected and grind, in the end, the color reaction and colorimetric estimation were carried out. The malondialdehyde contents were calculated by equation.

(3) The ascorbic acid oxidase activity were measured by residual titrimetric method (Bai *et al*, 1996): The normal laminas were used in the experiment. It was first cleaned and select 2.0 g in weight. Then the enzyme was extracted for activity analysis. In the end, the activity were calculated by equation.

# 2 Conclusion and analyses

## 2.1 Breeding season of *L. grandispicum* Y. J. Fei

The breading observation experiment were successively carried out from 1999 to 2001, The breeding properties are illustrated in Table 1.

**Table 1 Results of breeding of *L. grandispicum* Y. J. Fei**

| Items | Seeding | Seedling emergence | Tillow | Stem extension | Ear-bearing | Head sprouting | Blooming | Maturation |
|---|---|---|---|---|---|---|---|---|
| 1999~2000 | 24/10 | 31/10 | 17/11 | 25/2 | 28/3 | 6/4 | 11/4 | 24/5 |
| 2000~2001 | 4/11 | 14/11 | 12/12 | 1/3 | 30/3 | 8/4 | 13/4 | 26/5 |

From the above table, it can be seen that the growth season of *L. grandispicum* Y. J. Fei is the same as that of wheat. But its powerful tillow ability can reach about 20 per plant. And there were still a fraction of them in florescence when the wheat was harvested. In August, 2000, the summer seeding experiment of *L. grandispicum* and wheat in the test base have proved that: the result of seeding on August 1st was that *L. grandispicum* Y. J. Fei could not germinate which proved that the seed has dormant period and its winter habit is quite strong; The result of seeding on August 20th was that *L. grandispicum* Y. J. Fei could germinate and emergented the seedlings, but it could not bloom and seed the same as wheat in the same year. The *L. grandispicum* Y. J. Fei could mature living through the winter after vernalization in the second year, its breeding progress was the same as autumn seeding of wheat.

## 2.2 Chlorophyll content of leaf

During the blooming(15th of April, 2001), the chlorophyll content of leaf of blooming period of *L. grandispicum* Y. J. Fei and wheat were measured. The results are listed in Table 2.

**Table 2 SPAD and chlorophyll content**

| Items | EN1 | EN4 | *L. grandispicum* Y. J. Fei |
|---|---|---|---|
| $\overline{SPAD}$ value | 29.2 | 30.1 | 30.6 |
| Chlorophyll content ($10^{-3}$ mg/cm$^2$) | 18.25 | 19.00 | 19.43 |

Note: The results are the average means of 30 plants.

From Table 2, it can be seen that SPAD value of *L. grandispicum* Y. J. Fei and chlorophyll content are slightly more than EN1 and EN4.

The design principle of SPAD-502 chlorophyll trochometer is that the D-value of absorbancy a-

round 660 nm is measured by the lightening of electronic tube and the transmitting of leaf. SPAD chlorophyll trochometer was initially used to measure the leaf color, at present, the leaf color was applied to study the yield, quality and fertilization management of crops. Accordingly, SPAD chlorophyll trochometer is called leaf color trochometer and SPAD value is called leaf color value. The chlorophyll content can be worked out by the calculation of correlation function of chlorophyll content and SPAD value (Ai *et al*, 2000). The production of crop yield is the procedure of formation of organic substance that the light energy transforms to chemical energy through the chlorophyll in the plants. As a result, in the season of plant growth, the amounts of chlorophyll content of leaf play an important consequence on photosynthetic yields. Especially, in the later period of bearing, the amount of chlorophyll content is of great importance which the more amount of chlorophyll content are, the less degradation rate is and the longer function of crop has, the more potential of increasing in yield has.

**2.3 Malondialdehyde (MDA) content**

In the early stage (April 18th, 2001 ~ 19th, 2001) of milking of *L. grandispicum* Y. J. Fei and wheat, the malondialdehyde (MDA) content of the leaf next to last were measured. The results were shown as Table 3.

**Table 3　Malondialdehyde (MDA) content (μmol/g)**

| Items | Ⅰ | Ⅱ | Ⅲ | $\bar{X}$ |
|---|---|---|---|---|
| EN1 | 28.258 | 26.029 | 24.171 | 26.153 |
| EN4 | 33.118 | 33.758 | 28.766 | 31.881 |
| *L. grandispicum* Y. J. Fei | 23.406 | 19.789 | 20.211 | 21.135 |

From Table 3, it can be seen that malondialdehyde (MDA) content of the leaf next to the last of *L. grandispicum* Y. J. Fei is obviously lower than EN1 and EN4.

In the aging procedure of plant, the decreasing of superoxide dismutase (SOD) resulted in the increasing of free radical and the strengthening of lipid peroxidation resulted in the increasing of main oxidative product malondialdehyde (MDA) content. Namely, When the ageing was processing, a considerable decrease of SOD activity in the leaf and an increase of MDA cotent were observed (Lin *et al*, 1984; Cheng *et al*, 2000). It was obvious that the lower the MDA content was, the lower degree of ageing was and the longer function the leaf had. It can be inferred that the *L. grandispicum* Y. J. Fei is more resistant to ageing compared with both EN1 and EN4. It has been proved that the low degradation rate of leaf chlorophyll of *L. grandispicum* Y. J. Fei and its long function are in favour of the dry matter accumulation. It was observed from the field that when the leaf next to the last of wheat become to turn yellow, the leaf of *L. grandispicum* Y. J. Fei were still green that it was in favour of accumulation of photosynthetic product.

**2.4 Relationship between ascorbic acid oxidase activity and disease resistance**

During the early stage of milk filling (17th of April, 2001), the ascorbic oxidase activity of leaf of *L. grandispicum* Y. J. Fei and wheat were measured. The results are listed in Table 4.

**Table 4　Ascorbic acid oxidase activity**

| Items | EN1 | EN4 | *L. grandispicum* Y. J. Fei |
|---|---|---|---|
| Ascorbic acid oxidase activity(mg/g · min) | 0.632 5 | 0.467 5 | 2.145 |

From Table 4, it can be seen that ascorbic acid oxidase activity of *L. grandispicum* Y. J. Fei is much higher than EN1 and EN4. Ascorbic acid oxidase is a sort of oxydizing enzyme containing copper in plants and it is consisted in cytoplasm which is a sort of terminal oxidase in the respiratory chain. After the plant are violated by pathogenic bacteria, their respiration will strengthen and infectious organization are generally 10 times powerful than healthy tissue respiration (Heitefuss *et al*, 1998; Pan *et al*, 1995). As one type of terminal oxidase of respiration electron transfer, the increase of ascorbic acid oxidase activity can illustrate that the infectious plants contain excessive electron

flow in the respiratory action, which is in conform with the reinforcing of respiratory action and respiration can help the formation of phellogen around the wound. The quick wound healing of the plant can separate the healthy organization from the infectious parts so as to prevent infection spreading. The reinforcing of ascorbic acid oxidase activity is a sort of manifestation of the defending against pathogen infection of plant that is adapted to the enhancement of the respiratory action of diseased tissues. So a higher enzyme activity will result in a stronger disease resistance. From the above experimental results, it can be seen that *L. grandispicum* Y. J. Fei has quite strong disease resistance. And from the field observation, it was known that *L. grandispicum* Y. J. Fei do not have infectious status which is the same as actual measurement.

## 3 Discussion

The study reveals that *L. grandispicum* Y. J. Fei is highly resistant to aging and disease. The paper presented the preliminary study on *L. grandispicum* Y. J. Fei, but there are more issues waiting for further studies. For example, in the measurement of malondialdehyde content, only a certain time of malondialdehyde content was measured. In fact, for further study of degree of its resistance to aging, the malondialdehyde content should measured serially. Moreover, the extraction analysis of its isodynamic enzyme and DNA are still waiting for further studies.

In general, the preliminary study indicated that *L. grandispicum* Y. J. Fei is a sort of new germplasm resources. If the fine gene of *L. grandispicum* Y. J. Fei is transferred to wheat by the application of cell engineering, Chromosomal engineering and gene engineering, a genetic improvement of wheat is foreseeable.

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