Comprehensive Evaluation on Tolerance to Waterlogging Among Different Rapeseed Varieties

ZHANG Xuehua[2], JIANG Liangcai [1*], NIU Yingze [2], LI Haojie[1], PU Xiaobin [1], ZHANG Jinfang[1], JIANG Jun [1]
[1]*Crop Research Institute, Sichuan Academy of Agricultural Sciences, Chengdu, Sichuan, 610066, P. R. China*
[2] *Rapeseed Research Center, Sichuan Agricultural University, Ya'an, Sichuan, 625014, P.R. China*
Email: jlcrape@163.com

Abstract

Waterlogging tolerance of 100 rapeseed varieties were investigated by the method of testing related traits after germinated seeds anoxic stress. The results revealed that, compared with blank control, seed anoxic stress inhibited the growth of seedlings significantly; genetic variation was abundant in all of the six traits; coefficient of variation ranged from 23.66% (relative fresh weight) to 60.73% (RVI) among the 6 indexes. The RVI conform to normal distribution and varied from 0.04 to 0.83. Correlation analysis results suggested that very significant or significant positive correlation showed between indexes. Subordinate function analysis revealed that the subordinate value can represent the comprehensive waterlogging tolerance of rapeseed. Based on the subordinate value, we selected 10 waterlogging tolerance rapeseed varieties; we also found that waterlogging tolerance among three rapeseed species were different. Principle component analysis showed that according to cumulative contribution rate standard ≥ 85%, seedling vigor factor（λ_1=3.50）, seedling recovery growth factor （λ_2=1.20）and membrane stability factor（λ_3=0.71）were selected, the above three principle components account for 90.11% of total variations and covered most of the waterlogging resistance information. In conclusion, genetic variation was abundant in waterlogging tolerance among rapeseed varieties and some of them were resistant to waterlogged condition.

Key words: rapeseed, waterlogging tolerance, seed anoxic stress, subordinate function, principle component analysis

Introduction

Waterlogging will strongly influence seeds germination, seedlings growth, and reduce seedlings productivity, accelerate plants aging, lead to yield reduction. Waterlogging is one of the restriction factors regarding rapeseed yield reduction, may reduce yield by 17%-42.4% (Ouyang, 2001; Zhou et al., 1995; Gutierrez B. F. H., et al., 1996; Cheng, et al., 2003). Molecular markers, morphological parameters and physiological indexes may applied in identification of waterlogging tolerance germplasms. Based on waterlogging tolerance related traits of seedlings after germinated seeds anoxic stress, many researchers (Lu, et al., 1989; Fan, et al., 2005; Chen, et al, 2006; Zhang, et al., 2007) evaluated waterlogging tolerance of rapeseed, this method, to some extent, simulated field waterlogging at the beginning of rapeseed seeds distribution. Multivariate statistical methods such as principle component analysis, subordinate function (Bai, et al., 2008; Zhou, et al., 2001), were widely used in study on adverse circumstance tolerance of crops.

Materials and Methods

Experimental materials

100 rapeseed varieties (including 49 varieties in *Brassica napus* L., 28 varieties in *Brassia campestris* L. and 23 varieties in *Brassica juncea* L.) were investigated, they were mainly collected from western China.

Methods

Selecting rapeseed seeds which were fresh and healthy in a petri dish with 4 layers of moist filter paper at the bottom, and keeping the seeds under 25℃ for 24 hours to germinate, then storing them in 4 ℃ refrigerator for 36 hours, and selecting 50 germinated seeds whose radicles ranged from 0.2 to 0.5 cm in length, then three times of cleans with distilled water were carried out, and the seeds were put into a 10 mL plastic centrifuge tube and submerged in distilled water for 14h. After the treatment, testing the conductivity, then cleaning the seeds in distilled water for 3 times and moving them to a plastic box with 4 layers of moist filter paper at the bottom to keep growing under 25℃, the experiment repeated three times. The blank control was conducted without seeds submerging treatment. 6 days later, unit conductivity, relative seedling rate, relative root length, relative seedling length, relative fresh weight and vigor index (RVI) were calculated. SPSS17.0 was used for data analysis. Indexes were calculated with following formulae.

Unit conductivity（$mS\cdot cm^{-1}\cdot g^{-1}$） = $\frac{c}{w}$; Relative seedlings rate (RSR, 100%) = $\frac{SR_T}{SR_C}\times 100$;

Relative root length (RRL, 100%) = $\frac{RL_T}{RL_C}\times 100$; Relative seedling length (RSL, 100%) = $\frac{SL_T}{SL_C}\times 100$;

Relative fresh weight (RFW, 100%) = $\frac{FW_T}{FW_C}\times 100$; Relative vigor index (RVI) = $\frac{SR_T\times SL_T}{SR_C\times SL_C}$.

Where c is conductivity, w is seed dry weight, letters with subscript $_T$ is to symbolize value under treatment, letters with subscript $_C$ is to symbolize value under control. Subordinate value was calculated with $X_{(u)}=\frac{X-X_{min}}{X_{max}-X_{min}}$. When an index correlated negatively with waterlogging tolerance, we calculate the value with $X_{(u')}=1-\frac{X-X_{min}}{X_{max}-X_{min}}$.

Results

Variation analysis on waterlogging tolerance of rapeseed varieties

After anoxic treatment of germinated rapeseed seeds, seedlings growth was inhibited badly, average relative root length was only 26.63%, which suggested that the inhibition of root growth was the greatest. The result also indicated that RVI conform to normal distribution and vary from 0.04 to 0.83. Genetic variation was abundant in all of the six traits; coefficient of variation ranged from 23.66% (relative fresh weight) to 60.73% (RVI) among the 6 indexes. In simple comparison, waterlogging tolerance of three rapeseed species were different, relative seedling rate and relative root length of *B .campestris* L. were higher than the others, most indexes of *B. napus* L. were higher than the rest, every index of *B. juncea* L. suggest that waterlogging tolerance of the species was not so strong (table 1).

Correlation analysis on waterlogging tolerance of rapeseed varieties

Correlation analysis results (table 2) suggested that very significant positive correlation showed between relative vigor index and relative seedlings rate, relative root length, relative seedling length, relative fresh weight, very significant negative correlation showed between unit conductivity and relative seedlings rate, relative root length, relative seedling length, relative fresh weight, relative vigor index. Also, very significant positive correlation showed between relative seedling rate and relative root length, relative seedling length; very significant positive correlation showed between relative seedling length and relative fresh weight.

Table 1 Variation of waterlogging tolerance related indexes

Indexes	Mean				Variation Range	Range	CV/ %
	All	*B. napus* L.	*B .campestris* L.	*B. juncea* L.			
Unit conductivity/$mS\cdot cm^{-1}\cdot g^{-1}$	1.22	0.96	1.15	1.87	0.49～2.84	2.35	41.79
Relative seedling rate/ %	60.67	66.44	69.99	37.01	13.43～100.00	86.57	40.73
Relative root length/ %	26.63	28.84	31.14	16.43	5.96～66.84	60.88	46.92
Relative seedling length/ %	51.43	57.7	49.34	40.54	22.50～100.00	77.50	32.90
Relative fresh weight/ %	67.25	70.07	66.52	61.97	31.82～100.00	68.18	23.66
Relative vigor index	0.34	0.39	0.35	0.15	0.04～0.97	0.93	60.73

Table 2 Correlation of waterlogging tolerance related indexes

	Unit conductivity	Relative seedling rate	Relative root length	Relative seedling length	Relative fresh weight
Relative seedling rate	-0.52**				
Relative root length	-0.33**	0.74**			
Relative seedling length	-0.44**	0.34**	0.26*		
Relative fresh weight	-0.20*	0.23*	0.21*	0.67**	
Relative vigor index	-0.53**	0.65**	0.50**	0.88**	0.59**

Notes : *means the difference is significant at 5% level, **means the difference is significant at 1% level

Principle component analysis of waterlogging tolerance related indexes

Since correlation showed between indexes, we analyzed indexes through principle component analysis to find out independent ones, the results showed that according to cumulative contribution rate standard ≥ 85%, we chose three principle components which account for 90.11% of total variations and covered most of the waterlogging resistance information.

The first principle component account for 58.41% of total variation, and the characteristic value was 3.50. Maximum of characteristic vectors was relative vigor index; relative seedling rate came second; relative seedling length came third. In conclusion, we can name the first PC as seedling vigor factor. The second principle component account for 19.94% of total variation, and the characteristic value was 1.20. Characteristic vectors of 6 indexes gave the following subsequence (from great to little): relative fresh weight, relative seedling length, unit conductivity, et al. The second PC can be called as seedling recovery growth factor. The third principle component account for 11.76% of total variation, the characteristic value was 0.71. Characteristic vector of unit conductivity was far higher than the others. Since unit conductivity of germinated seeds dip solution showed the stability of cell membrane, the third PC was named membrane stability factor.

Subordinate function analysis on waterlogging tolerance of rapeseed varieties

Based on subordinate value, we selected 10 waterlogging tolerance rapeseed varieties, including 6 varieties in *B. napus* L. and 4 varieties in *B. campestris* L., and 6 of them are local germplasm rcsources. We also found that the maximum subordinate value was 0.78, which belong to a variety in *B. campestris* L.; the minimum subordinate value was 0.11, belong to a variety in *B. juncea* L.. Average subordinate value of varieties in *B. napus* L. was 0.53, in *B. campestris* L. was 0.49, in *B. juncea* L. was 0.28. We can conclude that waterlogging tolerance among three species of rapeseed were different, waterlogging tolerance of rapeseed varieties in *B. napus* L. and *B. campestris* L. showed far stronger than in *B. juncea* L..

Discussion

By the method of testing related traits after germinated seeds anoxic stress, we found that abundant variation existed among waterlogging tolerance of rapeseed varieties, this was consistent with the report of Fan（2005）, Chen（2006）, et al. Seedlings growth was inhibited after seeds anoxic treatment, especially the growth of root, this may related with water absorption. By the method of principle component analysis and subordinate function evaluation, we can make full use of the 6 indexes to evaluate waterlogging tolerance of rapeseed varieties; based on principle component analysis, we can conclude that RVI, relative seedling rate and unit conductivity can be used for preliminary evaluation on waterlogging tolerance of rapeseed varieties. Average subordinate value can represent waterlogging tolerance visually. Seeds germination might affected by many factors, such as seeds water content, storage time, seeds volume. Also, under RT, the uniformity of seeds is not so good. In our experiment, some improvements were set up, which can make the methods more effective, such as pretreatment of seeds under 4℃, selecting materials which were under the same condition. The result of this experiment was based on waterlogging related traits during early stage of rapeseed growth, the information is incomplete. Searching for more waterlogging related physiological indexes, and molecular markers should be considered more important in the following study.

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