Quantitative determination of the strength of rapeseed pod dehiscence*

TAN Xiaoli[1], ZHANG Jiefu[2], ZHANG Zhiyan[1], ZHOU Jia[1], JIANG Song[1], QI Cunkou[2], LI Jiayang[3]

[1]Institute of Life Sciences, Jiangsu University, Zhenjiang, Jiangsu Province, 212013, China;
[2]Institute of Industrial Crop, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu Province, 200014, China
[3]State Key Laboratory of Plant Genomics and National Center for Plant Gene Research, Chinese Academy of Sciences, Beijing,100101 China Email: xltan@ujs.edu.cn

Abstract

Rapeseed pods are easy to shatter causing yield loss during harvest. Furthermore the fragile pod is not suitable for mechanical harvest. Quantitive determination of strength of rapeseed pod dehiscence is necessary for studying the mechanism of rapeseed pod shattering and breeding cultivars with shattering tolerance. A "Ripping" method was developed to determine quantitively the pod dehiscence strength. To keep the different tests comparable, 6 pods per variety were balanced in a chamber at 25°C and at 50% relative humidity (RH) for 2 weeks, then the pods were enlaced with metalic thread at 2.5cm to the pedicel and glued onto a plate. The probe moved up to rip the pod, the maximal force that probe monitored was the pod dehiscence strength. Results of 47 tested varieties showed that the pod dehiscence strength among rapeseed lines varied greatly, hence selecting the pod shatter tolerant varieties among lines of oilseed rape for mechanical harvest was feasible. The "ripping" method of quantitive determination of the pod dehiscence strength had a potential value in rapeseed breeding and biological researches.

Key words: Quantitative determination, Pod dehiscence, Rapeseed,Ripping

Introduction

Rapeseed oil is the main source of the edible oil as well as the important industrial raw material and the future reproducible biomass energy. The pod dehiscence of rapeseed is formed in the process of long term evolution. At maturity, the pod is shattered to release the seeds to ensure extension of later generations, but it has the negative effect in rapeseed production nowadays. Due to the fragile pod of *Brassica napus* which is widely cultivated, yield loss in harvest is accounted for 10% of total yield. When the climate is worse during the harvest, this loss even reaches to 50% of the yield (Kadkol et al., 1984; Child and Evans, 1989; Price et al., 1996). The loss of seeds on the earth results in the contamination of subsequent crops. To avoid the seed loss caused by the pod shattering, early harvesting is often adopted, but this lowers seed oil content and increases seed chlorophyl content and eventually reduces the oil quality. Especially, the shattering pod does not fit to harvest mechanically and restricts the improvement of the production efficiency. Quantitively determining the strength of the pod dehiscence of different rapeseed varieties is prerequisite to understand the genetic and physiological mechanisms of pod dehiscence and select the varieties with shattering tolerance for rapeseed production.

The rapeseed breeders initially evaluate the pod dehiscence by weighing the seeds fell to the test plot after harvest (Kadkol et al., 1984). This method is valuable to evaluate the samples in the same place and the same year. With the change of test locations and time, the results from this approach would be varied and not be comparable. Other disadvantages of this approach is time consuming and laborious. Subsequently, bending pod method is developed, the pod is bended to crack and the angle which represent the pod dehiscence level when pod is shattered (Downey and Robbelen, 1989; He and Li, 1996). A low correlation is found between the maximal bending moment that the pod sustains before fracture and the percentage of shattered pods measured in field trials. The shattering angle is also affected by the pod length, diameter, moisture etc. And then the approach of VPS (Variable-speed pod splitter) is developed. The VPS is a mechanical device that provides a measure of the impact force required to trigger shattering of individual pod. Seed pods are released into the VPS at the different speed, and the speed breaking the pods is recorded. Pods remaining intact after impact at 60 Hz are considered to be non-shattering. The speed of the rotating spines is calibrated to convert Hz into revolutions per minute (RPM), and the result is reported in RPM (Timothy et al., 2003). Another quantitative method named Random Impact Testing is that 20 pods are placed together with six steel balls of 12.5mm diameter in a cylindrical container of diameter 20cm with its axis vertical. The container is then subjected to simple harmonic motion of frequency 4.98 Hz and of stroke 51mm in the horizontal plane. The pods are shaken for cumulative times of 10, 20, 40, 80s. At the end of each period, pods are removed from the container to examine the percentage of opened pods, the more percentage the opened pods, the more fragile the pods (Bruce, 2002). The two methods described above could quantitatively determine the pod dehiscence level, but the reproducibility is not good enough, and due to the nonstandard installation, the values could not be comparable among different places and time. The precise determination of pod physical character and energy release during pods shattering are reported, but the processes are too complex and the instrument are too expensive to use widely (Davies and Bruce, 1997; Morgan et al., 1998).

Here we report an approach of "Ripping" which has a good reproducibility and comparability to determine the pod dehiscence level. Method of Ripping can be widely used in shatter resistance breeding and the research on the biochemical and physiological mechanisms of pod dehiscence.

Quantitative determination of the strength of rapeseed pod dehiscence*

TAN Xiaoli[1], ZHANG Jiefu[2], ZHANG Zhiyan[1], ZHOU Jia[1], JIANG Song[1], QI Cunkou[2], LI Jiayang[3]

[1]Institute of Life Sciences, Jiangsu University, Zhenjiang, Jiangsu Province, 212013, China;
[2]Institute of Industrial Crop, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu Province, 200014, China
[3]State Key Laboratory of Plant Genomics and National Center for Plant Gene Research, Chinese Academy of Sciences, Beijing,100101 China Email: xltan@ujs.edu.cn

Abstract

Rapeseed pods are easy to shatter causing yield loss during harvest. Furthermore the fragile pod is not suitable for mechanical harvest. Quantitive determination of strength of rapeseed pod dehiscence is necessary for studying the mechanism of rapeseed pod shattering and breeding cultivars with shattering tolerance. A "Ripping" method was developed to determine quantitively the pod dehiscence strength. To keep the different tests comparable, 6 pods per variety were balanced in a chamber at 25°C and at 50% relative humidity (RH) for 2 weeks, then the pods were enlaced with metalic thread at 2.5cm to the pedicel and glued onto a plate. The probe moved up to rip the pod, the maximal force that probe monitored was the pod dehiscence strength. Results of 47 tested varieties showed that the pod dehiscence strength among rapeseed lines varied greatly, hence selecting the pod shatter tolerant varieties among lines of oilseed rape for mechanical harvest was feasible. The "ripping" method of quantitive determination of the pod dehiscence strength had a potential value in rapeseed breeding and biological researches.

Key words: Quantitative determination, Pod dehiscence, Rapeseed,Ripping

Introduction

Rapeseed oil is the main source of the edible oil as well as the important industrial raw material and the future reproducible biomass energy. The pod dehiscence of rapeseed is formed in the process of long term evolution. At maturity, the pod is shattered to release the seeds to ensure extension of later generations, but it has the negative effect in rapeseed production nowadays. Due to the fragile pod of *Brassica napus* which is widely cultivated, yield loss in harvest is accounted for 10% of total yield. When the climate is worse during the harvest, this loss even reaches to 50% of the yield (Kadkol et al., 1984; Child and Evans, 1989; Price et al., 1996). The loss of seeds on the earth results in the contamination of subsequent crops. To avoid the seed loss caused by the pod shattering, early harvesting is often adopted, but this lowers seed oil content and increases seed chlorophyl content and eventually reduces the oil quality. Especially, the shattering pod does not fit to harvest mechanically and restricts the improvement of the production efficiency. Quantitively determining the strength of the pod dehiscence of different rapeseed varieties is prerequisite to understand the genetic and physiological mechanisms of pod dehiscence and select the varieties with shattering tolerance for rapeseed production.

The rapeseed breeders initially evaluate the pod dehiscence by weighing the seeds fell to the test plot after harvest (Kadkol et al., 1984). This method is valuable to evaluate the samples in the same place and the same year. With the change of test locations and time, the results from this approach would be varied and not be comparable. Other disadvantages of this approach is time consuming and laborious. Subsequently, bending pod method is developed, the pod is bended to crack and the angle which represent the pod dehiscence level when pod is shattered (Downey and Robbelen, 1989; He and Li, 1996). A low correlation is found between the maximal bending moment that the pod sustains before fracture and the percentage of shattered pods measured in field trials. The shattering angle is also affected by the pod length, diameter, moisture etc. And then the approach of VPS (Variable-speed pod splitter) is developed. The VPS is a mechanical device that provides a measure of the impact force required to trigger shattering of individual pod. Seed pods are released into the VPS at the different speed, and the speed breaking the pods is recorded. Pods remaining intact after impact at 60 Hz are considered to be non-shattering. The speed of the rotating spines is calibrated to convert Hz into revolutions per minute (RPM), and the result is reported in RPM (Timothy et al., 2003). Another quantitative method named Random Impact Testing is that 20 pods are placed together with six steel balls of 12.5mm diameter in a cylindrical container of diameter 20cm with its axis vertical. The container is then subjected to simple harmonic motion of frequency 4.98 Hz and of stroke 51mm in the horizontal plane. The pods are shaken for cumulative times of 10, 20, 40, 80s. At the end of each period, pods are removed from the container to examine the percentage of opened pods, the more percentage the opened pods, the more fragile the pods (Bruce, 2002). The two methods described above could quantitatively determine the pod dehiscence level, but the reproducibility is not good enough, and due to the nonstandard installation, the values could not be comparable among different places and time. The precise determination of pod physical character and energy release during pods shattering are reported, but the processes are too complex and the instrument are too expensive to use widely (Davies and Bruce, 1997; Morgan et al., 1998).

Here we report an approach of "Ripping" which has a good reproducibility and comparability to determine the pod dehiscence level. Method of Ripping can be widely used in shatter resistance breeding and the research on the biochemical and physiological mechanisms of pod dehiscence.

Material and methods

Materials: All the materials were from the Institute of Industrial Crop, Jiangsu Academy of Agricultural Sciences, Including 47 lines of *Brassica napus*, and one line of *Brassica carinata*.

Instrument: Texture Analyser TAXT2-HD.

Methods: When pods turned to the light yellow, and began to dehydrate, about 10cm of middle inflorescence of 5 plants was cut and placed at 25°C, 50% RH for 2 weeks to keep the water content identical. Six pods each plant (2 pods each part of the upper, the middle and the lower on the inflorescence) were sampled. The pod was enlaced at 2.5cm to the joint point between the pod and the pedicle with the metallic thread and glued on the plate, then the replum was paralleled to the plate (Figure 1B).

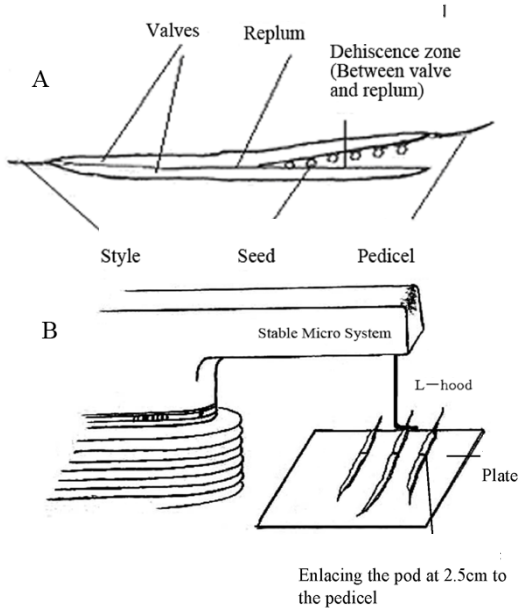

Fig 1. The structure of rapeseed pod and the installation of quantitative determination of the force for pod dehiscence.

A, Structure of a open pod. B, The installation of ripping method

Results and discussions

Installation of "Ripping method" to quantitatively determine the pod dehiscence strength

The rapeseed pod was composed of two valves, replum between two valves, dehiscence zone between the replum and the valve, style, pedicel and seeds attached to the replum. (Fig 1 A). At maturity, the pods began to dehydrate, and if the exterior forces such as mechanical, wind and so on, interacted with the pods, the valves and dehiscence zone detached resulting in the seeds release. Different varieties needed different exterior force to open the pods. We used the texture analyser to rip the rapeseed pod, and quantitatively determined the exterior forces necessary for pod opening. Before testing, all samples were put into a controlled room with 50% RH and at 25°C for two weeks. In Summer, the air-conditioned room could be easily controlled at 50% RH and temperature of 25°C. The pods with identical humidity were enlaced with metallic thread at 2.5cm to the end of pods to ensure that every test just determined the open strength at 2.5cm of the pod. Then, the pods were glued to a plate, and the replum was paralleled to the plate. One side of L-shaped hook was fixed in the probe of the Texture analyser, another side hooked the pedicel at the joint point of pod and pedicel (Figure 1 B). During the test, the plate was held in hands, the probe was moved upwards at the speed of 2 mm/min and the pod was opened. At the same time, the probe recorded the opened strength.

During the test, the probe drove the L-shaped hook moving forwards, the opening strength increased gradually before the pod opened. When the pod opened, the opening strength decreased rapidly. With the probe continued to move upwards, the opening strength increased slightly, and then decreased (Figure 2). The curve had a peak, the maximal opening strength appeared in 0.3s. The more the tolerant shattering, the higher the maximal value of opening strength. The peak value of the opening strength of each variety represented the pod dehiscence level. Fig 2 showed that the variety's pod dehiscence strength was 2.38 N. Here we used the precise and expensive instrument to determine the dehiscence strength, but in the rapeseed breeding practice, the maximal opening strength can be recorded by a cheap and simple spring balance instead of Texture Analyser.

Material and methods

Materials: All the materials were from the Institute of Industrial Crop, Jiangsu Academy of Agricultural Sciences, Including 47 lines of *Brassica napus*, and one line of *Brassica carinata*.

Instrument: Texture Analyser TAXT2-HD.

Methods: When pods turned to the light yellow, and began to dehydrate, about 10cm of middle inflorescence of 5 plants was cut and placed at 25°C, 50% RH for 2 weeks to keep the water content identical. Six pods each plant (2 pods each part of the upper, the middle and the lower on the inflorescence) were sampled. The pod was enlaced at 2.5cm to the joint point between the pod and the pedicle with the metallic thread and glued on the plate, then the replum was paralleled to the plate (Figure 1B).

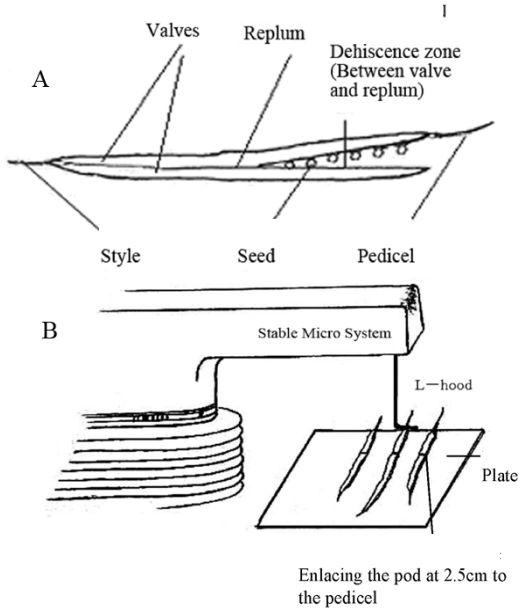

Fig 1. The structure of rapeseed pod and the installation of quantitative determination of the force for pod dehiscence.

A, Structure of a open pod. B, The installation of ripping method

Results and discussions

Installation of "Ripping method" to quantitatively determine the pod dehiscence strength

The rapeseed pod was composed of two valves, replum between two valves, dehiscence zone between the replum and the valve, style, pedicel and seeds attached to the replum. (Fig 1 A). At maturity, the pods began to dehydrate, and if the exterior forces such as mechanical, wind and so on, interacted with the pods, the valves and dehiscence zone detached resulting in the seeds release. Different varieties needed different exterior force to open the pods. We used the texture analyser to rip the rapeseed pod, and quantitatively determined the exterior forces necessary for pod opening. Before testing, all samples were put into a controlled room with 50% RH and at 25°C for two weeks. In Summer, the air-conditioned room could be easily controlled at 50% RH and temperature of 25°C. The pods with identical humidity were enlaced with metallic thread at 2.5cm to the end of pods to ensure that every test just determined the open strength at 2.5cm of the pod. Then, the pods were glued to a plate, and the replum was paralleled to the plate. One side of L-shaped hook was fixed in the probe of the Texture analyser, another side hooked the pedicel at the joint point of pod and pedicel (Figure 1 B). During the test, the plate was held in hands, the probe was moved upwards at the speed of 2 mm/min and the pod was opened. At the same time, the probe recorded the opened strength.

During the test, the probe drove the L-shaped hook moving forwards, the opening strength increased gradually before the pod opened. When the pod opened, the opening strength decreased rapidly. With the probe continued to move upwards, the opening strength increased slightly, and then decreased (Figure 2). The curve had a peak, the maximal opening strength appeared in 0.3s. The more the tolerant shattering, the higher the maximal value of opening strength. The peak value of the opening strength of each variety represented the pod dehiscence level. Fig 2 showed that the variety's pod dehiscence strength was 2.38 N. Here we used the precise and expensive instrument to determine the dehiscence strength, but in the rapeseed breeding practice, the maximal opening strength can be recorded by a cheap and simple spring balance instead of Texture Analyser.