In vitro screening stem rot resistant (tolerant) materials in *Brassica napus* L.

Shi Shuwen, Liu Lianghong, Wu Jiangsheng, Zhou Yongming

Plant Science and Technology College, Huazhong Agricultural University, Wuhan 430070, China

Stem rot (Sclerotinia sclerotiorum) is the most serious and widespread disease damaging rapeseed in China and the source of resistance to S.sclerotiorum has not been found so far. Thus rapeseed breeding against stem rot is very difficult. We attempt to create stem rot-resistant rape materials through the way of inducing mutation and in vitro screening with the oxalic acid toxin which is the pathogenic substance produced by S. sclerotiorum. Dry seeds of B.napus cultivar Huashuang 3 were exposed to gamma rays at doses of 400-4800Gy. The irradiated seeds were surface sterilized and cultured on B5 medium added the selection agent oxalic acid of 5.0 mM to conduct the first cycle screening. Survived plantlets (2 leaves stage) were transplanted into the B5 medium with 1.0-4.0 mM oxalic acid to treat for 4 days. The resistance (tolerance) of plantlets to stem rot was identified by inoculating the mycelia of S.sclerotiorum on leaves of plantlets screened. The results indicated that r-rays of 1600-4800Gy had inhibitory effect on seed germination and plantlets formation. In the treatment of 4800Gy, 42.5% of seeds germinated after 2 days of culture on B5 medium but 97.5% in check (not irradiated seeds). 7.3% and 99.2% plantlets were produced from the 1600 Gy and 0 Gy (control) treatments (no plantlets formed in 3200Gy and 4800Gy ones), respectively. Also, oxalic acid exhibited high toxicity to seed germination and seedling growth. Treating seeds with 5.0mM oxalic acid, seeds germination rate was only 3.9% of check. Percentage of survival seedlings was 100%, 80%, 2.6% and 0%, respectively by treating plantlets at 2 leaves stage using 1.0, 2.0, 3.0 and 4.0mM oxalic acid for 4 days. Therefore, it is concluded that the doses of 1200-1600Gy r-rays can be used to irradiate rape seeds for stress resistance screening in vitro; the concentrations of 5.0mM and 3.0mM oxalic acid is suitable for treating rape seeds and rape plantlets, respectively. The identification of disease resistance by inoculating mycelia in leaves showed that the size of lesion became small with increase of oxalic acid concentration used, the correlation coefficient = -0.9554. Additional, 9 plants tolerant stem rot were selected from oxalic acid treated tube seedlings regenerated from the seeds irradiated with the gamma rays of 400-1200Gy.