The Use of Oryzalin and Trifluralin as Substitutes to Colchicine in Chromosome Doubling.

Colchicine, oryzalin, and trifluralin are antimitotic agents that bind to the tubulin dimers during cell division and prevent the formation of microtubules and, thus, spindle fibers. (Peterson et al., 2003). While colchicine is by far the more popular product as it has been used since the 1930's to increase ploidy levels (Blakeslee and Avery, 1937), it is also very poisonous and considered hazardous material to ship. Two compounds, oryzalin (Fig. 1) and trifluralin (Fig. 2), have shown similar capability to induce chromosome doubling but without the potential dangers associated with the high toxicity of colchicine.

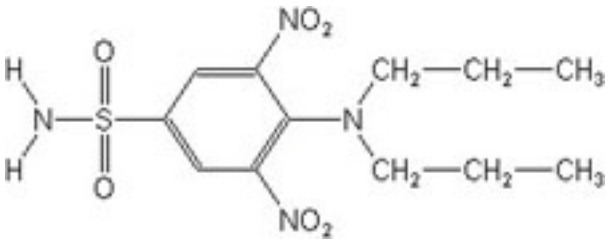

Fig. 1. Structure of oryzalin

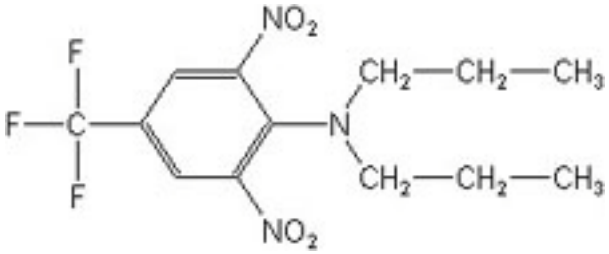

Fig 2. Structure of trifluralin

Oryzalin has been reported to double chromosomes in haploid clones of 3 pear cultivars at concentrations of 200 – 300 µM (Bouvier et al., 2002). Haploid apple shoots treated with oryzalin at 5 – 30 µM induced chromosome doubling better than colchicine at 0.25 – 1.25 mM (Bouvier et al., 1994). Oryzalin produced chromosome doubling in trihaploid kiwifruit (*Actinidia deliciosa*) shoots *in vitro* at 5 µM whereas colchicine was very toxic (Chalak and Legave, 1996). Interspecific diploid potato hybrids were induced to produce tetraploid shoots when apical buds were dipped for 24 hours in an 28.8 µM aqueous solution of oryzalin (Chauvin et al, 2004). In Rhododendron hybrids while oryzalin was more efficient at inducing polyploidy, plant survival was better with colchicine, presumably because diploids were more vigorous than the polyploids induced by oryzalin (Vainola, 2000). However, just the opposite was observed in the diploid rose 'Therese Bugnet' where tetraploid frequencies were highest after exposure of *in vitro* shoot tips to 5 µM oryzalin. Tetraploids showed thicker and darker green leaves, longer internodes, double the number of petals, and increased pollen viability (Kermani et al, 2003). Likewise, Ganga and Chezhiyan (2002) experienced positive results after oryzalin treatments to 4 diploid banana cultivars. They observed an increase in microshoot production of 4 banana cultivars when treated with 10 – 20 µM oryzalin relative to controls (no treatment). Colchicine required 125 – 200 times higher concentration to achieve comparable chromosome doubling but also had a negative effect on shoot generation.

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Microspores (immature pollen) of *Brassica napus* treated with 1 or 10 µM trifluralin during the first 18 hours of culture yielded doubled haploid embryos that developed and germinated normally (Zhao and Simmonds 1995). Later, Rudolf (1999) observed that 1.0 mg/L trifluralin treatment to cabbage (*Brassica oleracea* var. *capitata* L.) microspores increased the proportion of dihaploid plants. Microspore cultures of wheat have also yielded dihaploids after treatment with 0.1 – 10 µM trifluralin (Hansen and Andersen 1998). Trifluralin treatments of 0.086 – 0.0086% applied to the meristem of diploid *Rosa chinensis* seedlings resulted in polyploidization of the meristematic layers (L)I, LII, and LIII in varying percentages (Zlesak et al. 2005). All concentrations produced more diploidization that colchicine treatment where diploidization was observed to be confined to the LI layer.

Solution Preparation:

Oryzalin (Product No. O630) is an orange powder with a minimum purity of 97.5%; its formula weight is 346.36. While It is not readily soluble in water, it will dissolve quickly in DMSO yielding a clear solution with a yellow tint. DMSO solutions of oryzalin should be stored refrigerated. Note, DMSO will "freeze" at 18.5° C (65° F). DMSO will enhance penetration of oryzalin through plant tissue as well as skin so protective gloves should be worn when handling DMSO. Read the MSDS before handling DMSO. To prepare an aqueous stock solution of oryzalin it must first be dissolved in DMSO and then taken up to volume in water. The following is a representative protocol. No matter how a stock solution is prepared it is important to remember that the final concentration of DMSO in tissue culture medium should be limited to no more than 1 ml/L medium. This concentration has been for many years in many plant tissue culture applications. The preparation of aqueous stock solutions is tricky since oryzalin has such low solubility in water, approx. 2.6 mg/L. To prepare an aqueous solution the desired amount of oryzalin should first be dissolved in 1-2 mL of DMSO. Add this drop-wise to about 95% of the desired volume of *stirring* water. Since oryzalin has low solubility in water it should be dispersed quickly when it hits the water so that it doesn't fall out of solution, hence the suggestion to add it drop-wise to stirring water. Bring the final volume of water up to 100%. Since long-term solution stability has not been documented it is suggested that this be stored refrigerated for short periods or frozen in aliquots for longer term (months).

Trifluralin (Product No. T828) is also an orange powder with a minimum purity of 98%; its formula weight is 335.28. It is also soluble in DMSO but has very low solubility in water (< 1 mg/L). Solution preparation, appearance, storage, and DMSO precautions are the same as with oryzalin.

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