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Short communication

H_2O_2 induced seed viability assessment of Asian spider flower

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A reserve of viable, ungerminated seeds borne in a soil in a given habitat is called as 'seed bank'. Weed seed bank is the reserve of weed seeds present either on soil surface or scattered within the soil profile. It consists of both recently shed and older seeds that have persisted in the soil for several years. Most of the soil seed bank consists of buried seed, however some seeds lie on soil surface or in litter or humus. Such self-sown weeds appear simultaneously along with crops and result in strong crop weed competition causing reduction in grain/seed yield. It is essential to assess such seed reserves to judge their potential to cause harm to subsequent crop. Asian spider weed (*Cleome viscosa*) is a problematic weed of woodlands, grasslands, fallow lands, roadsides and waste lands. They grow on wide range of soils like sandy soils, calcareous and rocky soils and also found in dry and humid conditions. Knowledge of weed seed banks and their persistence is essential for long term weed management strategies.

Viability testing has been used to assess the viability of a wide variety of dormant weed seeds embedded in seed banks including many on agricultural weeds, restoration and conservation ecology and natural ecosystems, particularly those with frequent fires.Several methods have been used to estimate viability, germination test (counting the seedlings emerging from soil filled flats) (Forcella and Lindstrom 1988) and examination of imbibed seeds for signs of decay (Froud-Williams *et al*. 1984) as the indicators of seed viability of weed seed banks. Each test has advantages and disadvantages. *In situ* germination of soil borne seeds does not require separation of seeds from the soil, but it may require up to 2 years to induce germination of deeply dormant seeds (Roberts and Feast 1973). Typically, conditions are manipulated to encourage germination, but some seeds may still remain dormant and some seeds may die during the study. Tetrazolium testing provides a quicker way to obtain seed viability, saving time and labour. Reports on *Cleome viscosa* weed seed viability testing are scanty. Hence, the present investigation was carried out to know the viability of *Cleome viscosa*.

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Cleome viscosa seeds were collected from farmlands College and laboratory studies were conducted in the Department of Seed Science and Technology, Agricultural College and Research Institute, Madurai.

Twenty five seeds were soaked in water for 18 h in four replicates. The seeds were bisected longitudinal lateral and curved embryos extracted. Ten well formed embryos were incubated in darkness in 5 ml of 0.2% 2, 3, 5 tri-phenyl-tetrazolium chloride solution for 4 h then observed for staining pattern. This served as control.

Another batch of twenty five seeds was soaked in water for 18 h in four replicates and ten embryos were extracted (as detailed above) and soaked in 1% H_2O_2 followed by incubation in darkness for 15, 30 and 60 M followed by Tz staining for 4 h and observed for staining pattern.

All embryos were visually observed under the microscope (40x) for staining. The embryos soaked in 0.2% Tz solution remained unstained even after 4 h of incubation period (prolonged soaking after 20 h also did not stain the embryos). Whereas the embryos soaked in 1% H_2O_2 followed by 0.2% Tz solution showed stained embryos. The intensity of staining increased with increased soaking durations in 1% H_2O_2. The embryos soaked in 30 M showed prominent staining compared to 15 and 60 M. Being deeply dormant, untreated embryos did not stain. This was in agreement with Fontaine *et al.* (1994), who reported that exogenous application of H_2O_2 accelerates seed germination. H_2O_2 stimulates the respiration of deeply dormant embryos by acting as an electron acceptor in respiratory electron transport or by oxidizing endogenous inhibitors present in seeds leading to metabolism initiation (Henrotte Devillez 1976). Chien and Lin (1994) in cereals reported that during early phase of germination (imbibition phase), H_2O_2 may activate mitochondrial O_2 respiration and oxidative pentose pathway leading to the production of thioredoxin reduction by NADPH which mobilizes

the storage proteins present in seeds, resulting in promotion of seed germination. Fontaine *et al.* (1994) also proposed that H_2O_2 is helpful in cracking the hard seeds, allowing them to interact with water, thereby inducing the seed germination by stimulating metabolic reactions. Ogawa and Iwabuchi (2001) reported that endogenously generated H_2O_2 functions as a promoter of seed germination by oxidizing germination inhibitors present in seed. It could be concluded that mere soaking of *Cleome* embryos in Tz solution can not lead to staining misleading the observer about the viability status of the deeply dormant embryos. Hence, *Cleome viscosa* embryos must be activated by soaking in 1% H_2O_2 for 30 M followed by 4 h Tz staining for quick estimation of seed viability.

SUMMARY

Experiment carried out to assess the viability of Asian spider flower seeds revealed that presoaking embryos of *Cleome viscosa* in 1% H_2O_2 for 30 M followed by 0.2% tetrazolium (Tz) staining for 4 h showed pronounced improvement in staining compared to embryos not subjected to H_2O_2 that remained unstained.

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