A simple double-staining technique to assess seed viability in terrestrial orchids

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The need to estimate seed quality in seed-lots to optimise seed-banking efforts has stimulated researches for rapid methods to determine seed viability. Orchid seed quality is determined in various ways, especially by germination tests (even if time-consuming) and by viability tests, usually by Tetrazolium (TZ) staining. Terrestrial orchid seeds have an impermeable testa, so they require specific chemical scarification prior to vital staining to improve its effectiveness. Our results proved that the scarification protocol affects significantly both seed coat permeability and TZ results (2-way ANOVA, $P<0.0001$). In fact, eight different scarification methods resulted in TZ viability percentages ranging between 0 and 100% for the same seed-lot. Here, we report a rapid, simple-to-use protocol that can be used to test terrestrial orchid seed viability. Performing a permeability test by means of Trypan Blue dye, following the standard TZ staining, provides rapid information about seed coat permeability, colouring only the cells with corrupted membrane integrity. Viability can be calculated as the ratio between viable (bluish-to-blue testa and rose-to-red embryo) and permeable (bluish-to-blue testa) seeds so avoiding under-estimation of TZ results. Such double staining gives higher viability results than the simple TZ test, with a difference negatively correlated with the scarification effectiveness.