Characterization of the Semi-Permeable Layer in Seed of *Elymus nutans*

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Introduction

A semi-permeable layer of the seed coat exists in many species which allows controlled water uptake and gas exchange, while preventing solute transport (Beresniewicz *et al*. 1995). This layer could act as a barrier to apoplastic permeability and radicle emergence (Salanenka *et al*. 2009). It could also restrict the tetrazolium viability test and the electrical conductivity vigour test applied to evaluate seed quality (Yan and Wang 2008; Zhou and Wang 2012). An earlier study reported that semi-permeable layers exist in grass species, but recently research has shown that the layer was not found in seeds of oat (He 2011) or tall fescue (Yan 2008). This paper reports the location and chemical composition of a semi-permeable layer and its relationship to the electrical conductivity vigour tests in seeds of *Elymus nutans*, an important forage grass species widely distributed in Northwestern alpine grasslands China.

Materials and methods

The mature seeds were pre-soaked in distilled water at 20^{o}C for 24 hrs, then the intact seeds were placed into 2% (w/v) lanthanum nitrate solution and incubated at 20^{o}C for 24 hrs. The specimen sections were embedded in epon812 epoxy resin for transmission electron microscopy to test the location of the semi-permeable layer. Then the other specimens were embedded in Technovit 7100 epoxy resin for light microscopy and stained with 0.2% Sudan III and 0.05% aniline blue to identify the chemical composition of the layer. A range of different aged seed samples, having been either coat pierced or not (control) were subjected to the electrical conductivity test.

Results and discussion

A semi-permeable layer was found in seed of *E. nutans* located between the pericarp and seed coat. From outer to inner, the caryopsis coat could be clearly identified as pericarp, semi-permeable layer, seed coat, callose and aleurone layer under the light micrography (Fig. 1A). The semi-permeable layer resisted the lanthanum penetrating into the inner seeds, and the lanthanum was deposited as snowflake crystal outside of the layer. This layer was typically amorphous, highly compact, and easily distinguished under the TEM observation (Fig. 1B). The chemical composition of the layer was lipid as identified through Sudan III staining (Fig. 1A).

The conductivity value was significantly (P<0.05) correlated with gemination in seed samples of coat pierced, but not correlated in the control samples (Fig. 2). This result suggests that seed vigour of *E. nutans* is not able to be detected by the conductivity method because of the semi-permeable layer present.

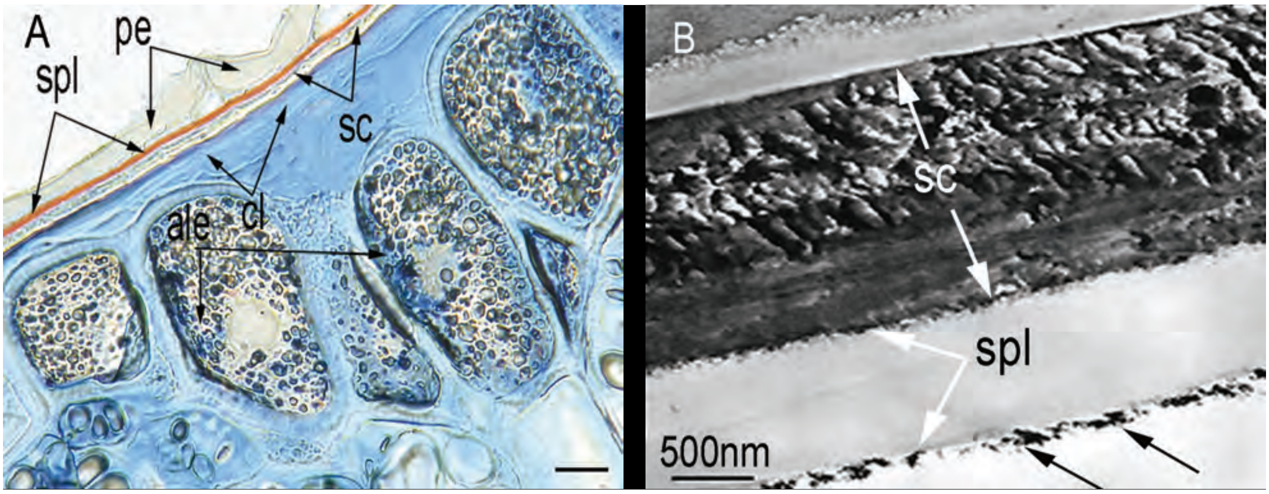

Figure 1. A: anatomy structures of caryopsis coat in *E. nutans*. B: ultrastructural investigation of caryopsis coat in *E. nutans*. pe: pericarp; sc: seed coat; cl: callose layer; ale: aleurone layer; sc: seed coat

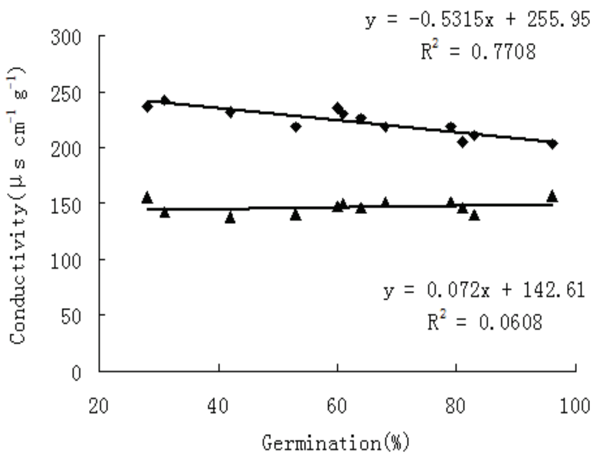

Figure 2. Correlation between germination and conductivity value in seeds with coat pierced ◆ and non coat pierced ▲

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