

Structural Characterization of the Anti-Oxidant Activity of the 1-Cys Peroxiredoxin Prx2 from the Resurrection Plant Xerophyta Viscosa

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Abstract

Xerophyta viscosa (Baker) is a monocotyledonous family Velloeciacea resurrection plant in South Africa, Lesotho, and Swaziland, which takes place in the summer rainfall. In open grasslands, it lives on the rocky terrain and often experiences water shortage cycles. It tolerates the loss of 95 percent of total cellular water as a resurrection plant and recovers the full metabolic competence within 3 days of rehydration. Members of this antioxidant group were previously only isolated from the monocotyledons in higher plants. Seed peroxiredoxins has been suggested to protect tissues against reactive oxygen during drying and early soaking and/or to sustain / protect during dormancy. Despite variations of grain growth in monocots and dicots, the direction of AtPer1 displayed an expression pattern similar to the dormancy-related transcript Per1 in barley during the seed production and germination. AtPer1 is established in situ as the first aleuron-specified transcript in development of Arabidopsis seeds in in-situ hybridization. It is often expressed in the embryo is the transcript. Seed expression of Per1 in an ABA-deficient mutant (*aba-1*) is unaltered whereas the seed expression of an ABA-insensitive mutant (*abi3-1*) is decreased and so AtPer1 expression is decreased. Throughout the vegetative tissue, transcript is not caused by ABA or drought as a result of stress. AtPer1 transcribed rates, however, abundance of AtPer1 transcripts is not sufficient to show dormancy in non-dormant mutants. This can be attributed to germination concentrations of the wildlife.

Key words: ABA-deficient mutant (*aba-1*), ABA-insensitive mutant (*abi3-1*), Dormancy, In-situ hybridization, Monocotyledons, Non-dormant mutants, Seed peroxiredoxins, *Xerophyta viscosa* (Baker).

Introduction

Resurrection plants are a rare subset of vegetative tissue desiccation tolerant plant species. Desiccation tolerance means that up to 95 percent of sub-cellular water can be submerged, sustained quiet in the dry state and complete metabolic activity recovery is possible within the hours or days of rehydration. Work has put plants as a single model to understand the mechanisms that is required to alleviate large water deficit stress and provide insights into mechanisms for improving drought tolerance strategies in crops in recent years. In an ever warming world, this is becoming ever more important for food security [1].

In most plants, the vegetative tissues are highly sensitive to water deficit, and lose viability when the total water content at maximum turgor is 41 to 70 % (depending on the species). Although there are many species which are common to the drought-prone regions (extreme

examples are succulent) that are capable in the face of drought of resisting water deficits, they usually grow slowly, with few suitable for human and domesticated animals consumption. Grains that constitute the majority of current annual food supplies are an inherently poorly developed ability to resist the loss of vegetative water and drought-caused crop loss [2]. In a study, the removal of the hydroxyl radicals was also shown. The first peroxiredoxin, which was isolated and characterized in yeast (TSA or antioxidant-specific thiol) has since become active in the species from bacteria to mammals.

The prediction of increased desertification in Australia, much of Africa, North, South and Europe over the next several years has been a result of the climate change models. In Australia, many of Africa, North and South America and Central Europe, with climate change model projections of increasing desertification for coming years; it is becoming important to establish the alternative strategies for agriculture under the intense environmental conditions. Till date, only partial success has been achieved in attempts to produce crops with a shortened period of growing and fruiting / seeding, and increased water-deficit resistance. The concept of this study is that crops with improved tolerance to water deficits will have greater value in future for improved food safety [3].

To this effect, for long periods, 135 angiosperm species which are commonly known as the resurrection plants have been recorded to survive the loss of up to 95% of the total amount of cell water in established tissues within the 24–72 hours after rehydration [4]. *Xerophyta viscosa* (Baker) (Fig. 1) is a single cell resurrection plant, which we used as a model to understand the vegetative water loss (drowning) tolerance mechanisms in order to make use of its certain properties in biotechnology of more drought-tolerant cereal crops.



Fig.1: *Xerophyta viscosa* chasmophytically in the Cathedral Peak Area of the Drakensburg Mountains, KwaZulu Natal, South Africa

For Per1, a barley 1-Cys peroxiredoxin whose expressions are restricted to the aleuron layer and embryo of developing seeds proposed that the desiccation resistance during the late stages of the seed development be given a special function. Protection from free radicals is an important factor of desiccation tolerance, and while in developing the seeds there are several other antioxidant mechanisms, including catalases, superoxide dismutases (SODs) and peroxidases, the most important protective functions that are not required for desiccating and germinating seeds. Only mid / late seed development, mature dry seeds, and early stages of soaking before germination symbols are present, in contrast with the Per1 single-copy gene expressed. Per1 is regulated environmentally similar to the late embryogenesis-abundant (Lea) transcripts which encode putative protection agents for dehydration. Per1 was actually classified as an ' atypical ' Lea gene [5].

The PER1 gene product is also involved in the dormancy regulation or in prediction of the dormancy conditions. This is based on the studies showing a positive correlation of the expression of barley peroxiredoxin with dormancy, a correlation that has also previously been identified in *Bromus secalinus* pBS128 for the Per1 homologue. Besides increased Per1 expression in soaked dormant seeds, transitory upregulation is observed in the soaked non-dormant seeds, thereby suggesting that the function of PER1 is not only associated with dormancy. PER1 should be effectively active, i.e. PER1 in vitro in a thiol-MFO system protects the DNA from degradation [6]. In imbibed dormant seeds, PER1 can be defensive, but a more theoretical hypothesis is that H₂O₂ may be extracted from planta, thus preventing the dormancy release. Although PER1 can be used to protect soaked dormant seed, a more speculative hypothesis is that H₂O₂ is eliminated in the plant, thus preventing the dormancy release.

Till date, 2-Cys peroxiredoxin group members have been isolated and characterized in the barley and spinach plants, as well as in *Arabidopsis* (Hv-bas1, So-bas1). Similarly, studies of the northern blots showed transcripts in several mono- and dicotyledons, which hybridizes to bas 1. Only one-cys-gene (barley; bromegrass; and rice) has been isolated from the monocotyledons in higher plants till date. That is why it has been undertaken to isolate and characterize the 1-cys peroxiredoxin gene from the dicot *Arabidopsis thaliana* in order to extend the studies on barley which have been begun [7]. *Arabidopsis*, a model plant that can be easily transformed with the seed dormancy, provides an excellent functional study system for further analysis of the different defensive, dormancy and germinative functions.

Overview of Taxonomy, Morphology and Distribution:

The genus, *Xerophyta* Jüss belongs to the family Velloziaceae which is found in Africa, Madagascar and the Arabian Peninsula. It consists of 45 species, out of which at least 10 are tolerant to drought. *Xerophyta viscosa* (Baker) occurs in the summer rains of African, subalpine and coastal pastures, where it lives off rocky grounds or inselbergs on the exposed grasslands and is typically found hanging on the cliff borders of South Africa, Lesotho and Swaziland (Fig. 2). Several *Xerophyta* species, such as *X. viscosa*, a composite of high altitudes in the wider Drakensberg range of South Africa is known for its plant diversity and

endemism, *viscosa* is found inside a rich-species Drakensberg Alpine Centre (DAC) [8]. While in these regions the mean annual precipitation (MAP) is high (often above 800 mm), the chasmophytic harsh environment of *X. viscosa* is low. Also during the rainy season, *X. viscosa* causes the extended periods of extreme water shortage.

The formation of chlorophyll-free aquiferous cells between the vascular bundles, lack of crystals in the leaves, and the presence of adaxial patellar leaf glands, is morphologically distinct from other desiccating resistant of xerophyta spp. (Fig. 3). Viscous metabolites such as velloziolone, manoyloxide, different diterpenes, phenolic compounds and fatty acids are secreted from these glands and can be used as the sunscreens in order to minimize the activation of light-chlorophyll during the initial drying phases. This process is known to produce extensive reactive oxygen species (ROS) that causes serious subcellular damage and loss of viability if not controlled. If elongated schlerophylatic leaves are present, minimal pliage can be used to decrease the surface area exposed to UV radiation through dehydration, thereby preventing the cellular damage as photosynthetic (chloroplasts and chlorophyll) is dismantled during the dehydration through its strategy for poikilochlorophyllous [9]. The exposed abaxial surfaces also exhibit an accumulation of violet anthocyanin pigment during dehydration which is considered to be a 'sun screen', reflecting photosynthesis activity during periods of rehydration before the complete chlorophyll breakdown [10]. Such re-synthesis and reparation of the damaged tissue and the resulting energy costs contributes to a longer recovery time than organisms with photosynthetic apparatus.

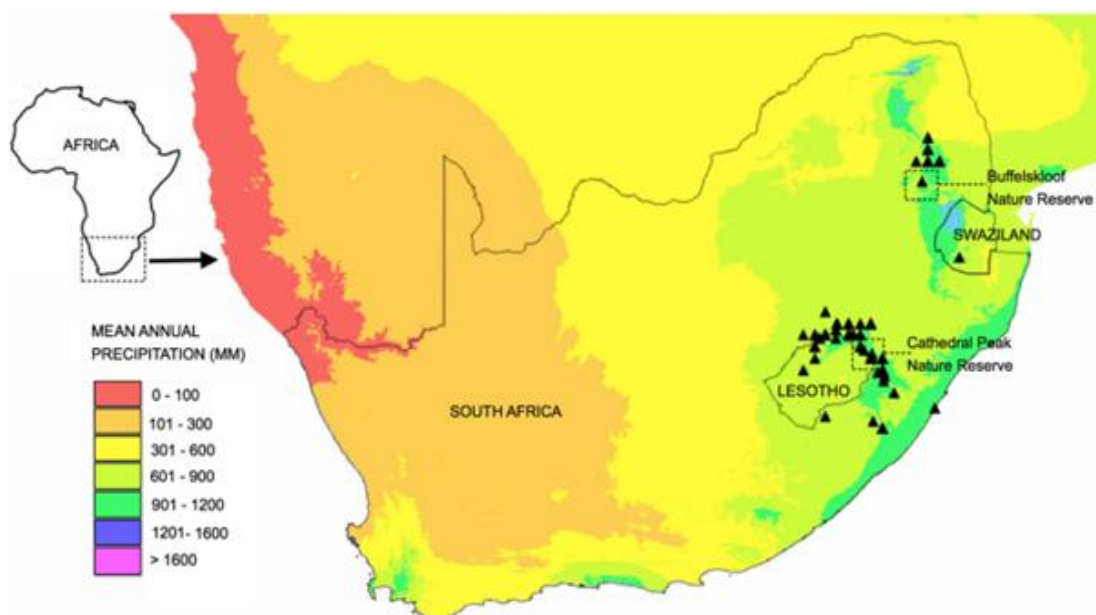


Fig.2: Distribution map of *Xerophyta viscosa* (Baker) in Southern Africa. Mean annual precipitation data for these areas are given. Collection sites are indicated by dashed squares.

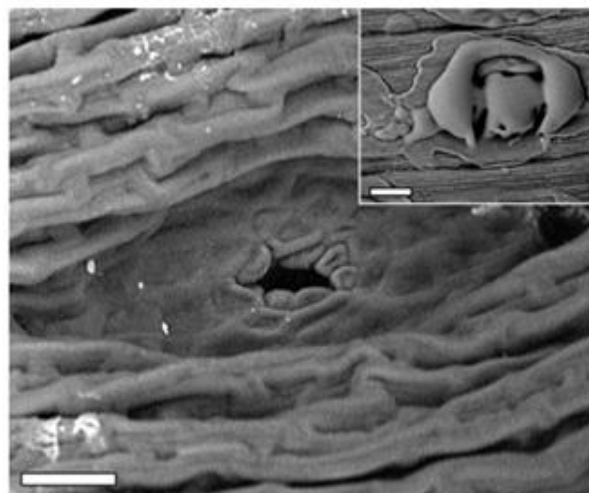


Fig.3: Scanning electron microscope image of the adaxial surface of a hydrated leaf of *X. viscose* showing a patellar leaf gland. The inset shows a gland covered by the sticky exudates that it produces. Scale bars represent 20 μm .

Plant collection, maintenance and methodology:

Plants collected in the natural reserve of Buffelskloof in nearby Lydenberg (Mpumalanga, South Africa) and the Nature Reserve of the Cathedral Peak in the mountains of Drakensberg (Kwazulu-Natal, South Africa) were researched. The plants at the University of Cape Town, South Africa, were maintained on the glasshouse conditions before dehydration and regeneration experiments that were necessary. The plants have been placed in a controlled ambient chamber for such experiments (16 h light, 350 $\mu\text{mol s}^{-1}\text{m}^{-2}$ -25 $^{\circ}\text{C}$; 8 h dark, 20 $^{\circ}\text{C}$; relative humidity of 50%); and allowed to acclimate at least two weeks in advance of the experiment. Plant dehydration was achieved via water containment up to an air-dry state of tissue water content (B5% water content as compared to the fully hydrated state). They have been kept in the dry condition for more than 1 week during which soil irrigation is used for rehydration. The first day of watering plants were well and soil for the remainder of the experiments was held waterproof. For the physiological and molecular studies referred to below, tissues were routinely examined during the drying and rehydration treatments. Among plants obtained from those different sites, there were no significant differences in response to desiccation.

Under these conditions, the dry air dehydration in the root and leaf tissue takes approximately 9 and 15 days, respectively. The roots of this mature plants species are recalcitrant in particular for the methodologies that are commonly used for the biochemical and molecular studies [11].

Conclusion

It is critical that plants resistant to the prolonged hot and dry conditions be made available to the African farmers, as projected that much of Sub-Saharan Africa is extended up to 2060.

For this understanding, plant *X viscosa* of the Resurrection acts as a guide. DT is a dynamic and is under the control of various interactive controllable phenomenon factors. In the absence of information from this genomic sequence (and any other resurrection plant species), we have used and defined the approach of the systems in which dehydration is followed by the physiological, biophysical, biochemical and molecular changes. Such functions have been listed in overcoming the major stresses linked to the continuous water deficit. While this strategy has provided a basis for understanding the key DT-related changes, there are pitfalls in this subtle but still they are the important changes.

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