Article

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Use of morphocolorimetric analysis to monitor germination success of hydroprimed seeds of *Coronilla valentina* subsp. *glauca* (*Fabaceae*)

Abstract

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This research aimed to investigate if the germination rate of *Coronilla valentina* seeds (*Fabaceae*) increased when subjected to extended hydro-priming. Analysis of hydroprimed seeds was carried out by using morphocolorimetric analysis to characterise the imbibition at different hydration time intervals. Seeds that had not been hydro-primed represented the untreated control. Seed swelling is a reliable and time-efficient method to measure seed imbibition, yet a poor predictor of seed germination success. Timing and temperature of the hydropriming treatment influence the germination rate of *C. valentina* subsp. *glauca*, with the optimum hydropriming treatment, was found to occur within a 72h period following the 75°C to 25°C treatment.

Key words: germination protocols, Mediterranean flora, morphometrics, hydropriming.

Introduction

The Mediterranean Basin is a biodiversity hotspot where many habitats are under threat and actions for habitat restoration are needed (Canals Ventin & Lazaro 2019). *Coronilla valentina* subsp. *glauca* (L.) Batt*,* the shrubby scorpion-vetch, is a perennial shrub with a patchy distribution in the Maltese Islands, where it forms an *Erico multiflorae-Coronilletum glaucae*, a typical high sea cliff garrigue phytosocial association with woody vegetation (Brullo & al. 2020). This shrub belongs to the Fabaceae family and its habitus stays low and wide, with the typical flower structure from the family, and glaucous green foliage. The characterisation of this subspecies is based on the low segment number of a dehiscent legume with a membranous stipule, less than 2 cm (Pignatti 1987). A critical stage of the plant life cycle is germination, and, as for many Fabaceae, *C. valentina* subsp. *glauca* seeds have a hard-coated testa which can prevent germination because their low permeability limits water uptake (Lambers & al. 2008). In real conditions, this results in limited population recruitment due to the low germination percentage, especially in its natural habitat characterised by calcareous soils (Escudero & al. 1996).

Hydropriming is the controlled hydration of seeds in water or a solution of low osmotic potential to initiate the germination metabolism before sowing (Singh & al. 2015). This simple and cheap technique improves seed water-uptake efficiency and is useful in areas with unpredictable abiotic stresses such as high temperature and drought (Sarfraz & al. 2019). During hydropriming, the seeds swell over a period of time as reflected in their change of volume and seed coat colour. The imbibition pressure will eventually lead to the rupture of the seed coat, causing a chain reaction of metabolism activation: starting with hydration of respiratory enzymes, renewed protein synthesis, the release of hydrolytic enzymes mobilizing stored reserves, cell enlargement and renewal of cell division (Lambers & al. 2008; Singh & al. 2015). However, excessive swelling may be an indication of over-imbibition which could lead to seed storage reserves deterioration and fermentation, eventually resulting in embryo death. Although seed swelling and color change are easy to characterise by visual observation, the use of morphocolorimetric methodology based on image analysis provides an accurate and quantitive approach, therefore providing objective and repeatable data (Vale & al. 2020).

This study aimed to investigate the efficiency of hydropriming treatment on seeds of *C. valentina* subsp. *glauca*. to increase the final germination percentage. The morphocolorimetric analysis is used to characterize the seeds of this subspecies and quantify the imbibition process over time.

Materials and methods

Accession data

Coronilla valentina subsp. *glauca* was identified according to Pignatti (1982) and circa 5000 seeds were collected in June 2021 in the Maltese Islands (WGS84: 35°49'18.4"N 14°27'36.4"E, 106 m a.s.l), in the locality of Wied Babu, part of the Rdumijiet ta' Malta S.A.C. (Natura 2000 site).

Treatment

Seeds were dried, manually extracted from their pod, cleaned and examined for infestation and macrocontaminants at the Seedbank facilities of the Department of Biology, University of Malta. Different conditions of the hydropriming treatment were tested using 2 temperatures and 4 time interval variables, plus a control group with no hydropriming and no temperature treatment (Table 1).

Each treatment was applied to 4 replicates of twenty-five seeds. Seeds were afterward sown in a compost: perlite (2:1) substrate and exposed to optimum germination conditions (fluctuating temperature 15 to 23.5°C, fluctuating relative humidity from 50 to 85%). Pots were watered by capillarity.

Data collection

The success of the treatment was recorded in daily germination emergence. Swelling of the seed due to imbibition was characterized by morphocolorimetric data using image analysis (Fig. 1) carried out with ImageJ 1.53 (Shneider & al. 2012) and the Particles8 plu-

Table 1. Variables of the Hydropriming treatment

Conditions (unit)	Value
Priming Time (hours)	0
	24
	48
	72
	96
Temperature (°C)	40°C constant
	75°C, then left to cool to 25°C

gin (Landini 2008). The image capture and analysis were based on the methodology detailed by Vale & al. (2020), with Hue, Saturation, and Brightness threshold value adjusted for the chosen blue background and seed color to increase contrast and therefore image segmentation accuracy. The settings used are described in the ImageJ macro, detailed in the Electronic Supplementary Material 1 (ESF1).

Data Analysis

Automatic classification of the seeds was carried out using k-means cluster analysis to identify 2 groups (Swollen and Not swollen, see Fig. 2). Explanatory variables were integrated into a PCA plot. Data analysis, including Kendall's τ correlation, was carried out using Past 4.07 (Hammer 2001).

Results

Field observations of specimens during seed collection match the description of *Coronilla valentina* subsp. *glauca* according to Pignatti (1982). This is the first recorded morphocolorimetric characterisation for seeds of this subspecies. A summary of the main morphocolorimetric variables is given in Table 2.

Germination dynamics of the replicates subjected to different treatments indicate a clear influence of timing and temperature parameters on germination success. Exposure to a constant 40°C decreased germination success over time, while the 75°C to 25°C treatment show an optimum reached at 72h hydropriming (Fig. 3).

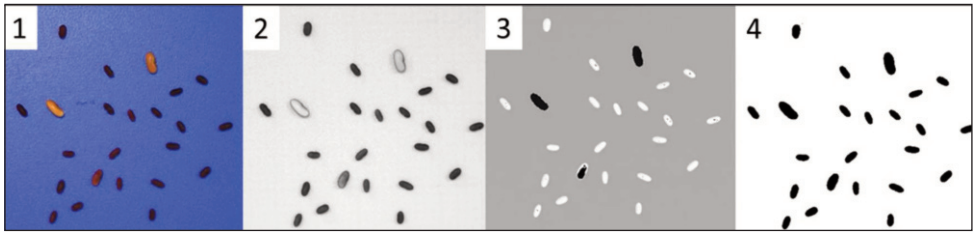

Fig. 1. Example of image segmentation using ImageJ: Original (1) Brightness (2) Hue (3) Mask (4).

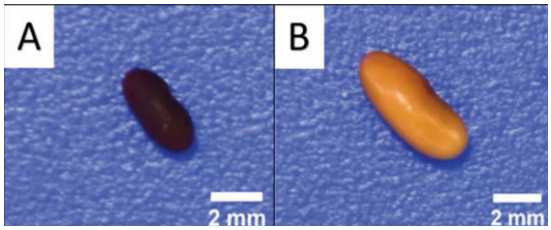

Fig. 2. Seed imbibition characterisation Not Swollen (A) and Swollen (B)

Table 2. Selection of morphocolorimetric variables extracted from N=500 seeds of *Coronilla valentina* subsp. *glauca.*

Variables	Measures				Shape index							Colorimetrics parameters		
	Perim	Area	Feret	Breadth	Circ	Roundness	Compactness	Solidity	Concavity	Convexity	Shape	RedAverage	GreenAverage	BlueAverage
unit	mm	mm²	mm	mm	N/A	N/A	N/A	N/A	N/A	N/A	N/A	None	None	None
Min	7.28	2.87	2.55	1.16	0.42	0.37	0.61	0.93	160.50	0.74	16.24	41.63	28.76	45.15
Max	11.12	5.47	3.75	2.06	0.77	0.70	0.84	0.99	807.00	0.95	30.20	93.89	52.82	69.64
Mean	8.87	4.09	3.27	1.57	0.65	0.49	0.70	0.98	321.25	0.90	19.34	55.60	37.19	57.39
Stand. dev	0.56	0.44	0.20	0.13	0.05	0.05	0.03	0.01	83.31	0.03	1.78	7.92	2.50	4.04

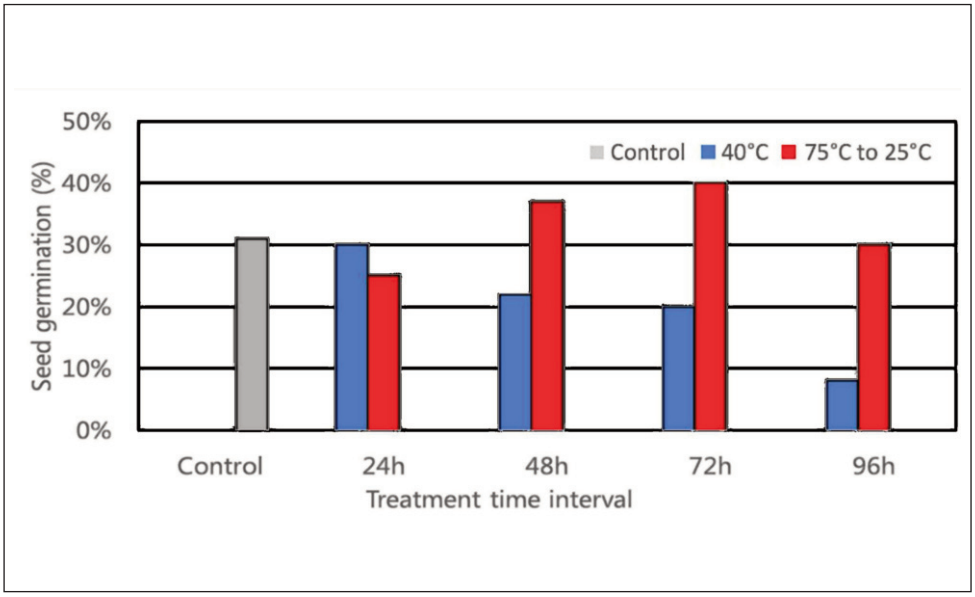

Fig. 3. Germination success according to variation in hydropriming pre-treatments conditions.

A Kendall's τ correlation was performed and show concordance between the count of swollen seeds obtained by a human observer and computer classification, $r(\tau) = .969$, $p < .0001$. Furthermore, the PCA plot displays a clear dichotomy of the 2 groups (swollen and non-swollen seeds) classified by the k-means clustering process. A Kendall's τ correlation shows a negative correlation between the count of swollen seeds and germinated seeds, at the end of the treatments. ($r(34) = -.347$, $p < .05$). The 2 first Principal Components of the analysis (PC1 and PC2) are plotted in Fig. 4 and account for 95.34 % and 4.11 % of the variability observed, respectively. The 3 most important loadings of PC1 are solely colorimetric indexes based on pixel value integrated density (RedIntDen, 0.82043; GreenIntDen, 0.50214; BlueIntDen, 0.27337) while the most important morphometric only parameter is the number of pixels forming the endocarp image (Pixels, 0.004012).

Discussion

Seed swelling characterisation using morphocolorimetric parameters is found to be a reliable and time-efficient method to measure seed imbibition, yet cannot be used as a pre-

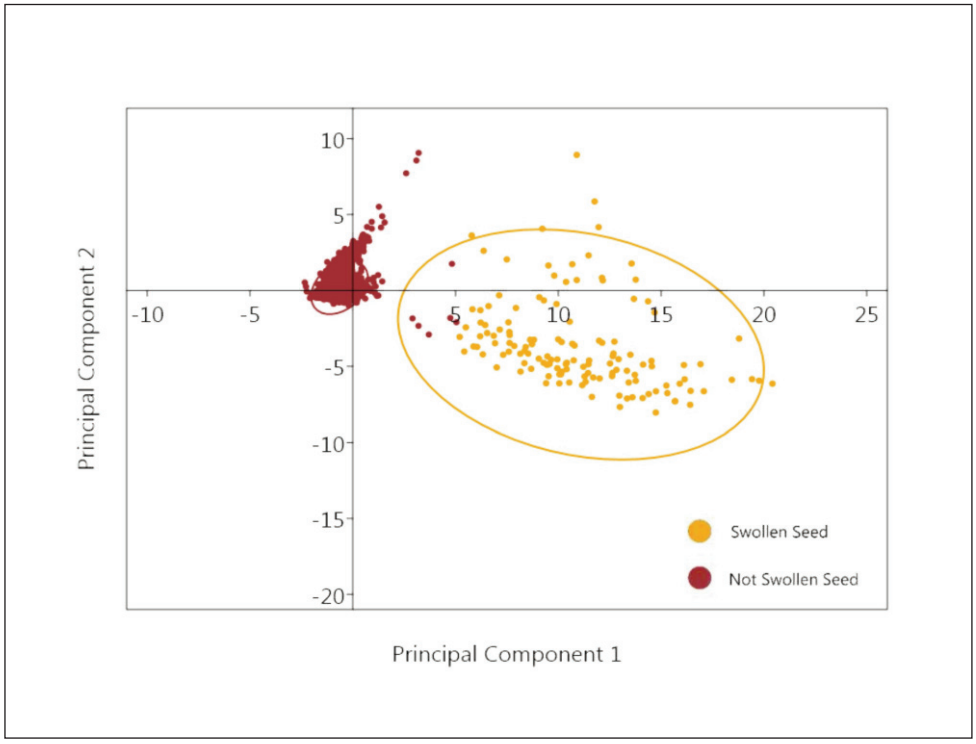

Fig. 4. PCA plot of the morphocolorimetric variations between swollen and non-swollen seeds, based on within-group variance-covariance matrix, with 95% ellipses.

dictor of seed germination success. The timing and temperature of the hydropriming treatment are found to influence the germination rate of *C. valentina* subsp. *glauca*, with the optimum hydropriming treatment, was found to occur within a 72h period following the 75°C to 25°C temperature treatment. This is the highest final germinate rate published to date for this subspecies, considering the results from Escudero & al. (1997).

The results presented in Fig. 3 show that appropriate temperature and timing help prevent over imbibition of the seed and ensure a higher germination rate. As expected due to imbibition, increased treatment time in both treatment conditions (40°C and gradual decrease from 75°C to 25°C) leads to an increased count of over-swollen seeds showing a decrease in germination success. Morphocolorimetric analysis of the seeds before treatment provides further characterisation of *C. valentina* subsp. *glauca* in the Maltese Islands and the methodology used for the Image analysis can be used in further studies of other species, including most of the Fabaceae (Lo Bianco & al. 2015; Vale & al. 2020). The results of the morphocolorimetric classification indicate that hydropriming treatment principally influences the color and the surface area of the endocarp while maintaining a consistent shape and external structure. All the seedlings produced were used for reintroduction into selected areas of disturbed Natura 2000 habitats in the Maltese Islands as part of the SiMaSeed habitat restoration project.

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