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ORIGINAL SCIENTIFIC PAPER

Chlorophyll fluorescence as a method for the prediction of germination success in common bean (*Phaseolus vulgaris* L.)

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Abstract

To explore the utility of chlorophyll fluorescence (CF) as a tool for the prediction of germination success we tested Croatian common bean landraces ('Trešnjevac', 'Biser', 'Zelenčec') differing in seed traits (length, width, height, weight) for seed germination. The significant differences in germination time were observed, landrace 'Biser' being the fastest to germinate. The univariate model including CF parameter F_v/F_m was not significant while the multivariate Cox model (seed length+F_v/F_m) had the highest concordance index. The utility of CF parameter F_v/F_m as a tool for the prediction of germination success is limited especially when other factors have a clear impact.

Keywords: common bean, landraces, germination, chlorophyll fluorescence (CF)

Introduction

Common bean (*Phaseolus vulgaris* L.) is of great agronomic interest worldwide and the most important grain legume for direct human consumption (Angioi et al., 2011). In Croatia, this crop is neglected and in danger of genetic erosion (Carović-Stanko et al., 2017).

Seed from different sources and with different weight may result in similarly high levels of germination under optimal conditions, but the same seed under the more stressful conditions in the field may have very contrasting abilities to establish plants due to differences in their vigour (Lima et al., 2005; Finch-Savage and Bassel, 2015). Seed viability is the ability of the embryo to germinate and is affected by a number of different conditions. Being able to predict seed viability is an important part of the planning process in agriculture (Shaban, 2013). Chlorophyll fluorescence (CF) is a rapid, non-destructive and inexpensive technique that has been used successfully in the evaluation of plant photosynthetic activity and it is seed sorting technique relying on measuring the amplitude of the CF signals of seeds (Gorbe and Calatayud, 2012; Kenanoğlu et al., 2016). It has been used in researches in common bean in leaves infected with bean rust (Peterson and Aylon, 1995), field screening for heat tolerant common bean cultivars (Petkova et al., 2001), as a marker for seed maturity and seed performance of *Brassica oleracea* seeds (Jalink et al., 1998), to assess seedling emergence potential and vigour of commercial tomato and cucumber seed lots (Demir et al., 2013) and to check seed germination performance of stored pepper seeds (Kenanoğlu et al., 2016). There are many different available Chl fluorometers and some of CF parameters that were used trough last few decades are F_0 (minimum fluorescence), Fm (maximum fluorescence), Fv/Fm (maximum quantum yield of photosystem II) etc. (Roháček, 2002; Gorbe and Calatayud, 2012).

The aim of this study was to test the seed germination of the three Croatian common bean landraces ('Trešnjevac', 'Biser', 'Zelenčec') differing in basic seed traits (length, width, height, weight), and to explore the utility of chlorophyll fluorescence as a tool for the prediction of germination success.

Material and methods

The research material included one hundred seeds of each of the three Croatian common bean landraces ('Trešnjevac', 'Biser', 'Zelenčec') multiplied and collected in a field trial at the University of Zagreb, Faculty of Agriculture, Department of Seed Science and Technology during the year 2018. The experiment was conducted in 2019. The seed length (mm), width (mm) and height (mm) of each seed was measured by Caliper and the weight (g) of each seed was measured by analytical balance.

The determination of seed chlorophyll fluorescence (parameters F_0 (minimum fluorescence) and F_v/F_m (maximum quantum yield of photosystem II), and chlorophyll fluorescence signal (Chl signal) was done using CropReporter® (PhenoVation, Wageningen, the Netherlands). Seeds were placed in a germination chamber under controlled conditions for germination following the recommendations of the ISTA (1993). Seeds were counted at regular time intervals every day to assess the number of germinated and nongerminated seeds.

The differences among landraces in seed germination were tested by survival analysis using Kaplan-Meier method with log-rank test (Kaplan and Meier, 1958) as implemented in R package 'survival' (Therneau and Grambsch, 2000; Therneau, 2015). The impact of landraces and seed traits (length, width, height, weight) on germination success were tested by Cox proportional-hazards model (Cox, 1972) using 'coxph' function from R package 'survival' (Therneau and Grambsch, 2000; Therneau, 2015). The same procedure was used to test the prediction accuracy of the chlorophyll fluorescence parameter F_v/F_m.

Results and discussion

Results of Kaplan-Meier analysis with log-rank test indicated that there were significant differences ($P < 0.05$) among three Croatian common bean landraces ('Trešnjevac', 'Zelenčec', 'Biser') in germination success. Landrace 'Trešnjevac' with the largest seeds had the maximum mean germination time (2.19 days), whereas 'Zelenčec' had medium seeds and mean germination time 1.82 days, while 'Biser' with the smallest seeds had the shortest mean germination time (1.41 days). Similarly, a Cox regression model for germination success that included landraces as a source of variation was significant ($\chi^2 = 20.92$; $P < 0.05$). In comparison to landrace 'Biser' used as a reference (Hazard Ratio = 1), 'Trešnjevac' reduced the germination success by a factor of HR = 0.43 (or 57%) while 'Zelenčec' by HR = 0.59 (41%) (Table l).

Table 1. Germination success expressed in terms of regression coefficients (β), Hazard Ratios (HR) with 95% confidence intervals (CI) and *P* values (Wald test), as estimated using a Cox regression model with time to germination as time scale. The model compares germination of seeds of the landrace 'Trešnjevac' and 'Zelenčec' to seeds of the landrace 'Biser' as reference (HR = 1.00).

Landrace	β	HR	CI (95%)	P_{Wald}
'Trešnjevac'	-0.836	0.43	0.33-0.58	***
'Zelenčec'	-0.530	0.59	0.44-0.78	***

*ns – non-significant; *significant at P < 0.05; **significant at P < 0.01; ***significant at P<0.001*

A series of separate univariate Cox regression models that included four seed traits size (length, width, height, weight) and chlorophyll fluorescence parameters revealed that only parameter F_v/F_m was significant while all the seed traits had a significant impact on germination success. In all cases, the negative β coefficients indicated that all the variables had a negative effect of germination success. For every 1-unit increase (mm) of seed length, width and height, the germination success decreased by the factor of HR = 0.89 (11%), HR = 0.73 (27%) and HR = 0.76 (24%) in relation to overall germination success while for every 1-unit increase (g) of seed weight germination success was reduced by the factor of HR = 0.13 (87%). However, the values of F_v/F_m were from 0.16 to 0.33 and these values failed to predict germination success (Table 2.)

Table 2. Germination success as influenced by seed size (length, width, height, weight) or predicted by chlorophyll fluorescence parameter F_v/F_m, as estimated using separate univariate Cox regression models with time to germination as time scale.

Variable	β	HR	CI (95%)	P_{Wald}
Seed length	-0.121	0.89	0.85-0.92	***
Seed width	-0.322	0.73	0.62-0.85	***
Seed height	-0.277	0.76	0.68-0.84	***
Seed weight	-2.028	0.13	0.06-0.28	***
F_v/F_m	-3.433	0.03	0.00-1.12	ns

*β - regression coefficient; HR - Hazard Radion; CI (95%) - confidence intervals; P_{Wald} - significance of the Wald test (ns – non-significant; *significant at P < 0.05; **significant at P < 0.01; ***significant at P<0.001)*

The use of F_v/F_m in prediction of germination success was further explored by multivariate Cox regression models that included F_v/F_m values with the four seed traits. Two out of four models gave significant results for F_v/F_m (Table 3) and had a higher concordance index (defined as a fraction of correct predictions) in comparison to the same models without F_v/F_m (Table 4). The best model included seed length and F_v/F_m and it had a concordance index of 0.750. However, there were no significant differences in concordance indices of the six best models presented in Table 4.

Table 3. Multivariate Cox regression models for germination success in which chlorophyll fluorescence parameter F_v/F_m gave significant *P* values

Model	Variable	β	HR	CI (95%)	P_{Wald}
1	Seed length	-0.124	0.88	0.85-0.92	***
	F_v/F_m	-3.720	0.02	0.00-0.87	*
2	Seed weight	-2.059	0.13	0.06-0.27	***
	F_v/F_m	-3.631	0.03	0.00-0.97	*

*β - regression coefficient; HR - Hazard Radion; CI (95%) - confidence intervals; P_{Wald} - significance of the Wald test (ns – non-significant; *significant at P < 0.05; **significant at P < 0.01; ***significant at P<0.001)*

Table 4. Goodness-of-fit of the six best Cox regression models for germination success as compared using concordance index (C-index; standard error: SE) defined as a fraction of correct predictions. *P* values were obtained by comparing models to the best one (Seed length + F_v/F_m) using Z-test.

No.	Model variables	C-index	SE	$P_{Z\text{-}test}$
1	Seed length + F_v/F_m	0.750	0.025	-
2	Seed height	0.745	0.024	ns
3	Seed weight + F_v/F_m	0.742	0.025	ns
4	Landrace	0.736	0.023	ns
5	Seed length	0.736	0.026	ns
6	Seed weight	0.728	0.026	ns

In concordance with previous studies (Borji et al., 2007; De Ron et al., 2016), the results indicate that landraces with larger seeds take more time to germinate probably because it need more time for water absorption. The utility of F_v/F_m in prediction of overall germination success is generally limited although it could improve the concordance of the models that include some other seed traits.

Conclusion

There are significant differences among Croatian common bean landraces ('Trešnjevac', 'Biser', 'Zelenčec') in germination time most likely due to differences in basic seed traits (length, width, height, weight) as all the traits have a significant impact *per se* on germination success. It seems that the utility of chlorophyll fluorescence parameter F_v/F_m as a tool for the prediction of germination success is limited especially in cases in which other factors have a clear impact on germination time.

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Fluorescencija klorofila kao metoda za određivanje klijavosti sjemena graha (*Phaseolus vulgaris* L.)

Sažetak

Kako bi se utvrdila korisnost fluorescencije klorofila (CF) kao alata za predviđanje klijavosti sjemena, testirana je klijavost hrvatskih tradicijskih kultivara graha ('Trešnjevac', 'Biser', 'Zelenčec') koji se razlikuju u svojstvima sjemena (dužina, širina, debljina, težina). Uočene su značajne razlike u vremenu klijanja pri čemu je 'Biser' najbrže klijao. Univarijantni model, koji je uključivao parametar fluorescencije klorofila F_v/F_m, nije bio značajan, dok je multivarijantni Cox-ov model (duljina sjemena+F_v/F_m) imao najviši indeks podudaranja. Korisnost parametra fluorescencije klorofila F_v/F_m kao alata za predviđanje klijanja je ograničena, posebno u slučajevima kada i drugi čimbenici imaju utjecaj.

Ključne riječi: grah, tradicijski kultivari, klijanje, fluorescencija klorofila