IECAG 2021

Proceedings

Assessment of the fall dormancy of Lucerne (*Medicago sativa* L.) in the Mediterranean area †

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† Presented at the 1st International Electronic Conference on Agronomy, 3–17 May 2021; Available online: https://sciforum.net/conference/IECAG2021

Abstract: The difficulties in assessing the distinctness characters in lucerne plants have recently led to reject the registration to the EU database of plant varieties for several new varieties with valuable agronomic characteristics. The tendency of lucerne plants to grow during winter and their fall dormancy can be efficient discrimination tools for the varieties during registration tests. The dormancy class of many Italian varieties is still unknown. The aim of our study was to validate the method proposed by the International Union for the Protection of New Varieties of Plants (UPOV) for the assessment of the dormancy class and to classify an adequate number of Italian cultivars to be used as control varieties in future registration tests. The experiment was carried out during three consecutive Distinctness, Uniformity and Stability (DUS) trials, in Italy. The method was based on the linear regression analysis to evaluate the functional relationship between the fall dormancy class (FDC) and the natural plant height (NPH) measured during the different DUS trials in five growth stages. A Principal Component Analysis (PCA) was performed on the NPH values of lucerne varieties. The first principal component and the fall dormancy class had a significant linear relationship in all the trials. NPH4 and NPH15 showed to be a useful tool for the discrimination of lucerne cultivars with different dormancy ratings. Our experiment confirmed that the dormancy rating is an important characteristic for discrimination of lucerne varieties.

Keywords: Lucerne, Alfalfa (Medicago sativa L.), Fall Dormancy, Distinctness test

Citation: Faria, J.M.S.; Vicente, C. Essential oils and volatiles as nematodicides against the cyst nematodes *Globodera* and *Heterodera* †. *Proceedings* **2021**, *68*, x. https://doi.org/10.3390/xxxxx

Published: date

1. Introduction

The difficulties in assessing the distinctness characters in lucerne plants have recently led to reject the registration to the EU database of plant varieties for several new varieties with valuable agronomic characteristics [1]. The tendency of lucerne plants to grow during winter and their fall dormancy (FD) could be an efficient tool for discriminating varieties during registration tests and an interesting agronomic characteristic to evaluate cultivar suitability to different climatic conditions [2]. The information about the dormancy of lucerne are limited and the dormancy class of many Italian varieties is still unknown.

The aim of our study was to validate under Mediterranean climate the method proposed by the International Union for the Protection of New Varieties of Plants (UPOV) for the assessment of the dormancy class and to classify an adequate number of Italian cultivars to be used as control varieties in future registration tests.

2. Materials and Methods

The experiment was carried out at the experimental farm of CREA-DC located in Palermo, Italy (38.08° N, 13.42° E; 34 m a.s.l), during three consecutive Distinctness, Uniformity and Stability (DUS) trials (18 months each; 2016-2019).

Seven varieties, representing the fall dormancy classes (FDC) 2, 4, 5, 6, 7, and 9, were used as control varieties, while other twenty-one varieties under DUS testing were evaluated to assess their dormancy class. Natural plant heights (NPHs) was measured during the different DUS trials according to UPOV guidelines in five growth stages: 2 weeks after the first autumn equinox following sowing (NPH2); 6 weeks after the first autumn equinox following sowing (NPH3); about 1 month after the beginning of growing the year after sowing (NPH4); 2 weeks after the second autumn equinox following sowing (NPH14); 6 weeks after the second autumn equinox following sowing (NPH15). A cut was made 2 weeks before and 2 weeks after each autumn equinox (Figure 1).

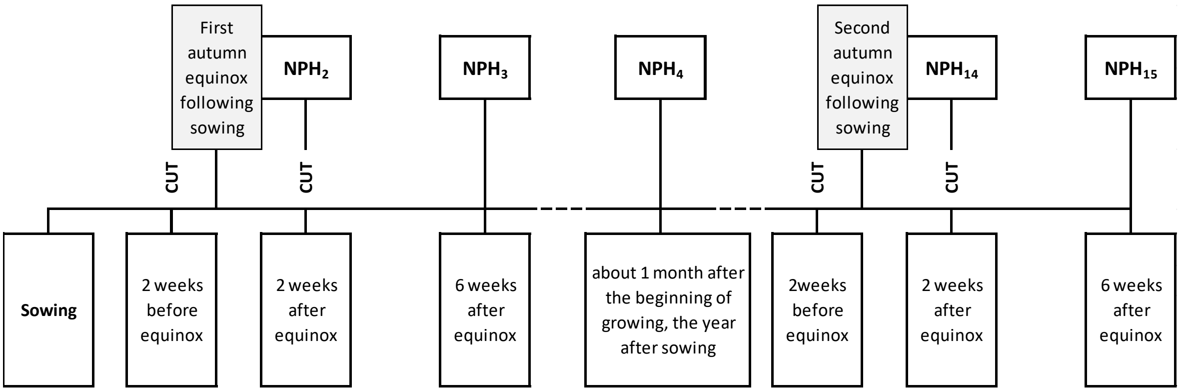

Figure 1. Time of year for the measurements of natural plant height in each DUS trials.

The NPH values measured for control varieties were used to evaluate the FDC by applying the models proposed by Montegano [3] and Teuber [4]. The first method uses regression analysis to fit a linear equation to the PC1 values obtained from a Principal Component Analysis (PCA) performed on NPHs. Thus, a PCA was performed for each DUS trial, investigating the correlation among the varieties and the natural plant height at different growth stages. The input matrix for the analysis comprised the NPH values of the control varieties. Regression analyses were then performed to fit a linear equation to the data of PC1 from the PCA of each DUS trial [3]. Moreover, regression analyses were also performed to fit a linear equation to the data of each NPH [4]. The linear regression models obtained from the single NPH measurements over the three DUS trials were compared to find the equation with the highest correlation. The equations obtained were then used to estimate the dormancy rating of the varieties under DUS testing that was compared to the dormancy assessed visually by using the control varieties as references.

3. Results

The biplots obtained from the PCA related to each DUS trial showed that NPH_4 and NPH_{15} were positively correlated with PC1, while NPH_2 was mainly correlated with PC2.

In each DUS trial, three main groups could be distinguished based on the fall dormancy class of the control varieties: Dormant (FDC 1-3), Intermediate (FDC 4-6) and Non-Dormant (FDC>6). (Figure 2).

The PC1 and the fall dormancy class had a significant linear relationship in all the years of the trial (Figure 2).

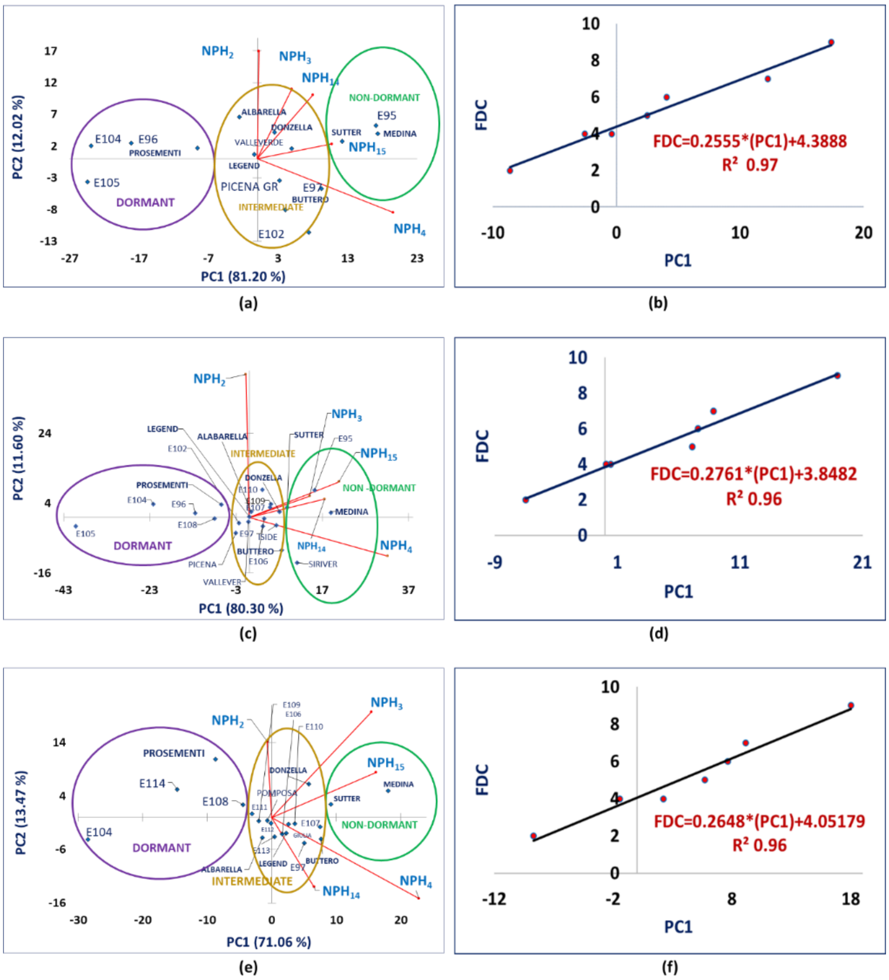

Figure 2. Biplot of Principal Component Analysis of the each DUS trial: (a) 2016-2017; (c) 2017-2018; (e) 2018-2019 and Linear Regression Models (b) 2016-2017; (d) 2017-2018; (f) 2018-2019.

These models showed R^2 values of 0.97 in the first DUS trial and 0.96 in the second and third trial. As regards the models obtained from the regression analyses between the FDC of control varieties and each NPH a high and significant correlation with FDC (R^2 values higher than 0.72) was found using NPH_4 and NPH_{15} (Table 1).

Table 1. Linear regression models obtained using NPH values.

DUS Trial	NPH_n	Model	r	R^2	Pr > F
2016 - 2017	NPH_2	$FDC=1.4616*NPH_2-6.2439$	0.16	0.02	0.738
	NPH_3	$FDC=5.968*NPH_3-37.606$	0.46	0.22	0.295
	NPH_4	$FDC=3.1414*NPH_4-11.053$	0.99	**0.99**	**< 0.0001**
	NPH_{14}	$FDC=6.3167*NPH_{14}-45.849$	0.63	0.40	0.128
	NPH_{15}	$FDC= 6.1215*NPH_{15}-34.403$	0.85	**0.72**	**0.015**
2017 - 2018	NPH_2	$FDC=-1.3607*NPH_2+15.168$	-0.21	0.05	0.644
	NPH_3	$FDC=7.7577*NPH_3-45.271$	0.85	0.72	0.016
	NPH_4	$FDC=4.0021*NPH_4-20.389$	0.94	**0.88**	**0.002**
	NPH_{14}	$FDC=12.364*NPH_{14}-85.999$	0.73	0.53	0.064
	NPH_{15}	$FDC=7.0959*NPH_{15}-38.689$	0.97	**0.94**	**0.000**
2018 - 2019	NPH_2	$FDC=-0.3903*NPH_2+8.3198$	-0.06	0.00	0.905
	NPH_3	$FDC=5.2886*NPH_3-32.3865$	0.74	0.54	0.059
	NPH_4	$FDC=2.9213*NPH_4-9.6057$	0.90	**0.82**	**0.005**
	NPH_{14}	$FDC=4.3468*NPH_{14}-31.763$	0.48	0.23	0.273
	NPH_{15}	$FDC=7.2351*NPH_{15}-47.19$	0.91	**0.83**	**0.004**

Table 2 shows the estimated FDC classes of the control varieties calculated from the models with PC1, NPH4 and NPH15. The linear regression models obtained using PC1 fitted better, showing lower differences between estimated classes and official FDC of the control varieties (Table 2).

Table 2. Estimated FDC classes of the control varieties using the models with PC1, NPH4 and NPH15.

		DUS trials 2016 - 2017			**DUS trials 2017 - 2018**			**DUS trials 2018 - 2019**		
Control Varieties	Official FDC	Estimated FDC			Estimated FDC			Estimated FDC		
		PC1	(NPH_4)	(NPH_{15})	PC1	(NPH_4)	(NPH_{15})	PC1	(NPH_4)	(NPH_{15})
PROSEMENTI	2	2.2	1.9	2.5	2.1	2.5	2.9	1.7	1.1	3.5
ALBARELLA	4	3.7	3.6	3.5	4.0	3.9	4.5	3.7	5.1	2.9
LEGEND	4	4.3	4.4	5.0	3.9	4.0	3.2	4.6	5.3	4.8
DONZELLA	5	5.0	5.1	4.5	5.8	6.4	4.4	5.6	5.3	5.2
BUTTERO	6	5.4	6.1	7.1	5.9	5.5	5.8	6.1	6.2	5.1
SUTTER	7	7.5	7.2	7.7	6.3	5.8	7.1	6.5	6.2	6.5
MEDINA	9	8.8	8.7	6.7	9.1	9.1	9.1	8.8	7.9	9.2

This model (FDC=a*PC1+b) was then used to estimate the FDC of the 21 varieties under DUS testing and the results are presented in Table 3.

Table 3. Estimated FDC classes of the varieties under DUS testing.

Varieties	FDC	Group	Varieties	FDC	Group	Varieties	FDC	Group
E104	1	Dormant	E112	4	Intermediate	GIULIA	5	Intermediate
E105	1	Dormant	PICENA GR	4	Intermediate	E110	5	Intermediate
E114	1	Dormant	E102	4	Intermediate	E97	5	Intermediate
E96	2	Dormant	E113	5	Intermediate	E107	6	Intermediate
E108	2	Dormant	E109	5	Intermediate	ISIDE	6	Intermediate
E111	4	Intermediate	E106	5	Intermediate	SIRIVER MK II	7	Non-dormant
POMPOSA	4	Intermediate	VALLEVERDE	5	Intermediate	EM95	9	Non-dormant

4. Discussion and Conclusions

The results of this study allowed: (I) the validation of the method proposed by UPOV guidelines to our environmental conditions; II) the selection of a fair number of control varieties to be used in future official DUS tests.

Our study confirmed that the dormancy rating obtained through the NPHs is an important characteristic for the discrimination of lucerne varieties. The models obtained from PC1, NPH4 and NPH15 explained a high proportion of variability. In our climatic conditions, high temperatures occurring in early autumn don't allow discrimination among the varieties by using NPH2. Therefore, the NPH2 measure could be avoided, reducing the costs of DUS tests of lucerne.

In conclusion, the results of this work could help to simplify the assessment of variety dormancy ratings.

Author Contributions: Conceptualization, B.F., C.M.; Data curation, B.F., V.A., M.V.; Formal analysis, B.F., C.M.; Investigation, B.F.; V.A., M.V.; C.M; Methodology, B.F.; C.M.; Supervision, B.F., A.M. and C.M.; Validation, B.F.., A.M. and C.M.; writing—original draft, F.V., A.M. and C.M.; writing—review and editing, B.F.; A.M., C.M.. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgements: Natale Accomando and Leonardo Fricano for the active contribution to the agronomic management of the experimental fields.

Conflicts of Interest: The authors declare no conflict of interest.

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