NARDI FUNDULEA, ROMANIA
www.incda-fundulea.ro

ROMANIAN AGRICULTURAL RESEARCH, NO. 33, 2016
Print ISSN 1222-4227; Online ISSN 2067-5720

USE OF NORMALIZED DIFFERENCE VEGETATION INDEX (NDVI) FOR ESTIMATING GENOTYPIC DIFFERENCES IN WHEAT SEEDLINGS RESPONSE TO WATER STRESS INDUCED BY GRADUAL DRYING OF THE SUBSTRATE

Monica David

National Agricultural Research and Development Institute Fundulea, 1 N. Titulescu st., 915200 Fundulea, Calarasi County, Romania

E-mail: inismona35@gmail.com

ABSTRACT

Drought and osmotic stresses, that can both affect the plant in any vegetation stage, including seedling stage, may be characterized through water status measurements. Non-destructive remote sensing offers a viable alternative for screening many genotypes in a short time. Significant genotypic differences were previously demonstrated in seedlings growth response to water stress, but characterizing growth necessitated frequent measurements, which limited the usefullness of this method in routine screening of breeding material. In an attempt to avoid this limitation, we searched for genetic differences between wheat cultivars in normalized difference vegetation index (NDVI) in seedlings exposed to water stress through gradual drying.

17 wheat genotypes, contrasting in drought resistance, were subjected to water stress by gradual drying of the substrate, during 27 days, using 1000 cm^3 of soil, initially soaked with 300 ml of water. The cultivars were tested under controlled conditions in a growth chamber. We found significant differences between wheat genotypes for NDVI, as well as significant correlation between NDVI measured in water stress seedlings and osmotic adjustment estimated by pollen test, as well as between NDVI and an estimation of yield response to drought in the field. NDVI measured in seedlings exposed to gradual drying of the substrate can allow exploring and test a large number of genotypes and can be used for off-season direct comparison between cultivars.

Key words: water stress, drought tolerance, gradual drying, seedlings, high throughput, precision phenotyping, osmotic adjustment.

INTRODUCTION

Drought resistance has become a high priority in many wheat breeding programs, especially in the context of climate changes. Morgan (1988) suggested that coleoptile and seedling roots length response to water stress can be used to identify differences for osmoregulation among wheat genotypes. His results, along with other studies (Saulescu et al., 1995), opened prospects of using seedling tests that could be informative about the behaviour of adult plants under water stress.

Seedling tests in controlled conditions can respond to the necessity of short duration and rapid assessments of drought response in breeding programs. They also have the advantage of low cost and good control of the stress, and can detect differences in the first stage of plant development, allowing enhanced selection.

For drought resistance, the intensity of green colours for wheat leaves can be good indicator of plant water status, being able to give an indication on osmotic adjustment capacity (Hackl et al., 2013). Using a GreenSeeker instrument we can take quick measurements for: vegetative green biomass, canopy photosynthetic capacity, LAI, GAI, biomass, nutrient content and the results can provide predictions of yield, biomass accumulation, growth rate, soil coverage, early vigour, and abiotic stress detection (Reynolds et al., 2012).

Preliminary results indicated that "Seedling response to water stress induced by gradual drying of the substrate" can be used as a simple method to approximate drought resistance in wheat (David, 2013). In that study

Received 7 October 2015; accepted 10 December 2015; First Online: March, 2016. DII 2067-5720 RAR 2016-165

we used frequent measurements of seedling growth to characterize the response of genotypes under stress (Figure 1), because leaf elongation is one of the plant processes most sensitive to water stress conditions (Hsiao, 1973; Hsiao and Acevedo, 1974; Cutler et al., 1980). The role of water in leaf elongation is thought to be mediated by turgour potential, which interacts with cell wall properties to determine the rate of expansion (Lockhart, 1965; Cutler et al., 1980).

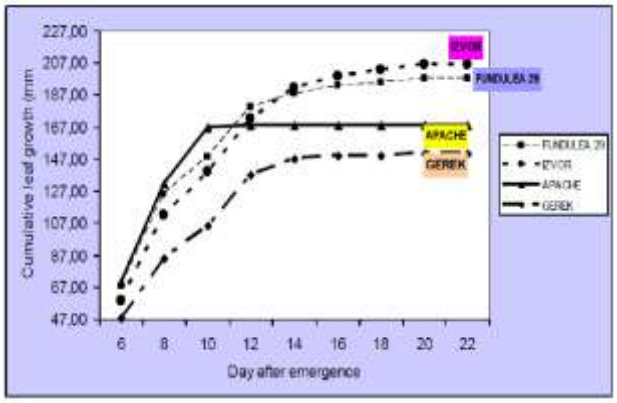

Figure 1. Leaf cumulative growth of 4 genotypes on gradual drying substrat (David, 2013)

The present research had the purpose to establish if using NDVI in such a test, could successfully replace frequent growth measurements, increasing this way the efficiency of screening genotypes for osmotic adjustment.

MATERIAL AND METHODS

17 wheat genotypes, contrasting in drought resistance, were subjected to gradual drying during 27 days, using 1000 cm^3 of soil initially soaked with 300 ml of water. The seeds were allowed 2 days to imbibition, at 2°C and 3 days for germination at 24°C. Then the seeds were transferred to the substrate, seedlings growth was in uniform light (12.000 lux), at temperature 24°C for day and 16ºC for night, and 80% humidity. We used 3 replications of each genotype. We planted 16 germinated seeds for each pot. Pots were randomized every day till the end of the study. The photoperiod regime was 16 hours of light and 8 hours of darkness. In comparison with the 2013 test (David, 2013), we changed the pot type and increased the soil volume and the number of seedlings per pot for ensuring the development of the desired stress level (Figure 3).

As seen in Figure 3, the changes in the pot type, soil and water volume and number of seedlings per pot did not change the response to water stress of the seedlings.

Normalized difference vegetation index (NDVI) represents the light reflectance of measurements in the red and near infrared (NIR) spectrum regions.

Figure 2. Seedlings from growth chamber in 6th day of the study

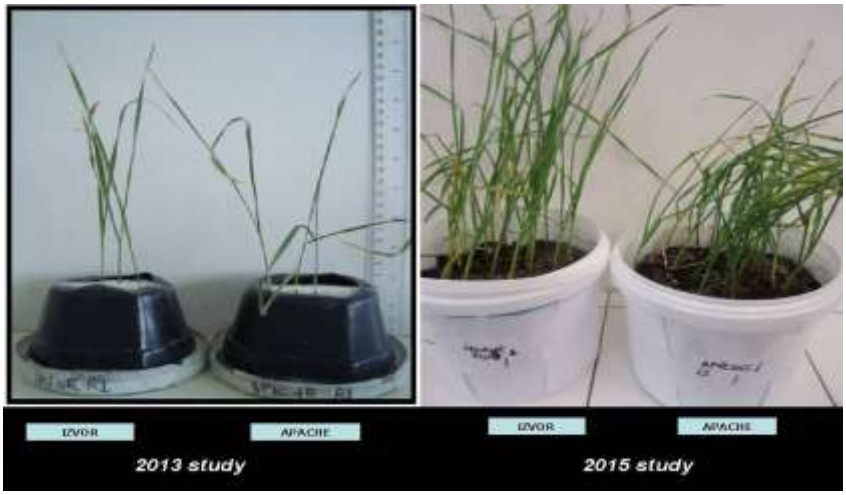

Figure 3. Comparative reaction to water stress between 2 contrasting genotypes

The measurements for NDVI were realised at equal distances from seedlings (45 cm – distance between instrument and seedlings), laterally from the leaves with the aid of manufactured support for the Greenseeker instrument placed at 18 cm (distance between manufactured support and experimental pot) to each analyzed pot. The data average for one replication is represented by 3 values taken through averages of 10 instrument records on the same single experimental pot (Figure 4).

Figure 4. Measurements taken by GreenSeeker Handheld Crop Sensor (Trimble) on wheat seedlings

Statistical analyses – ANOVA and correlations between different indicators – were conducted using the software Excel on 3 replications of each genotype.

RESULTS AND DISCUSSION

a. Variance analysis for NDVI

Table 1. Variance analysis for NDVI

NDVI (Day)	*F*	*F crit*	*P-value*
23	**64.90481**	1.915321	6.87E-22
27	45.96987	1.915321	2.47E-19

The analysis of variance indicates significant differences between genotypes analyzed on days 23th and 27th (Table 1) for this indicator. The maximum amplitude of variation for NDVI was obtained in this interval (0.299; 0.153) (Table 2).

On average of days 23 and 27, the variety that had the highest NDVI value was Izvor (0.299). In contrast, in cultivars Apache, Romulus, Fundulea 133 and Turda 81, NDVI values in seedlings were between 0.162 and 0.153 (Table 2). On day 23, the same variety, Izvor had the highest NDVI value, while the lowest values were also recorded for varieties Apache, Turda 81, Romulus and Fundulea 133.

Table 2. Data averages for NDVI in wheat seedlings analyzed on days 23th and 27th

Genotypes	Data averages for NDVI
Izvor	0.299
Otilia	0.281
Dropia	0.261
Faur F.	0.257
Dor	0.255
Alex	0.246
Glosa	0.243
Fundulea29	0.241
Delabrad	0.237
Bezostaia	0.231
Fundulea 4	0.230
Boema 1	0.220
Odesskaya 51	0.189
Apache	0.162
Fundulea 133	0.159
Romulus	0.157
Turda 81	0.153

b. Correlation between NDVI and osmotic adjustment estimated by pollen test under drought

Morgan (1988) used coleoptile and seedling root length response to water stress to identify differences for osmoregulation among wheat genotypes. Direct mesurements of osmotic adjustment for the cultivars included in our experiment were not available, but five of the cultivars analysed in this study were tested in the field for osmotic adjustment, by the indirect pollen test (David, 2012). We found significant positive correlation between osmotic adjustment estimated by pollen test and NDVI (r=0.87*) measured in seedlings exposed to water stress. Variety Izvor had the best values for osmotic adjustment capacity expressed by pollen test and for NDVI indicator, while the variety with the worst values for this indicator was Apache (Figure 6).

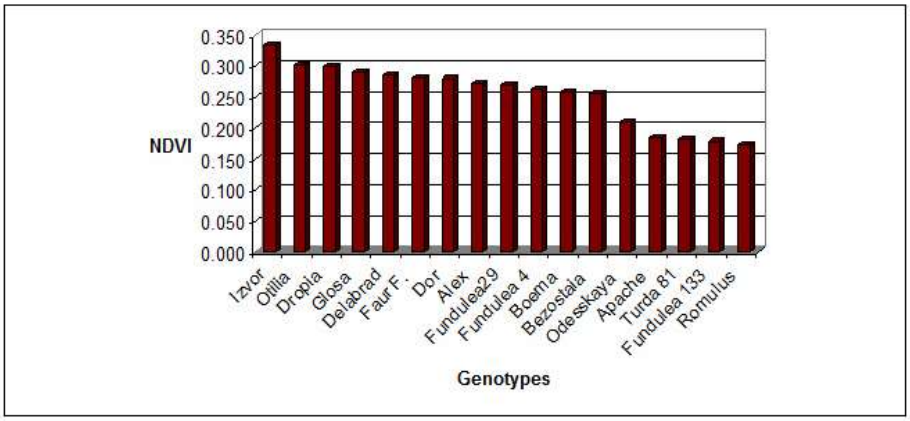

Figure 5. Cultivars ranking on day 23th for NDVI

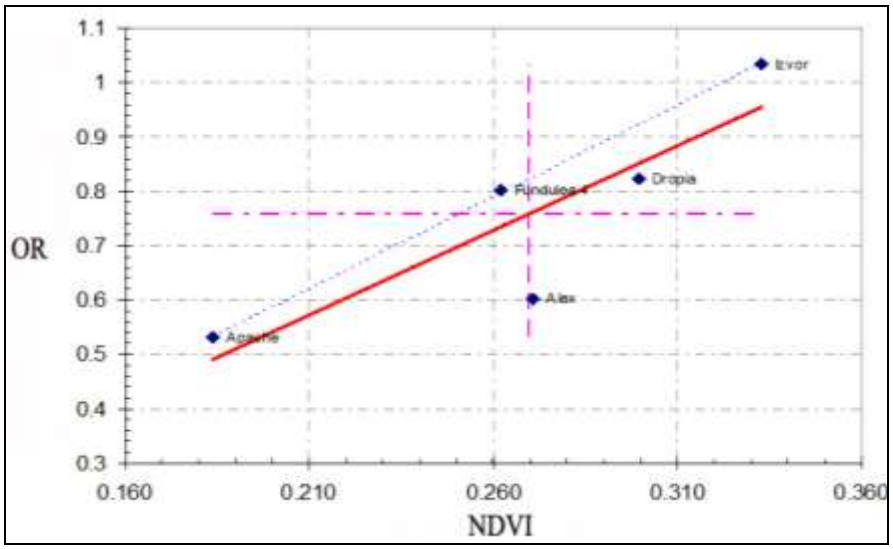

Figure 6. The relationship between induced osmotic adjustment (KCl) estimated by pollen test and NDVI on day 23th

c. Correlation with field performance

Estimates of seedling response under water stress can be useful only if they correlate with genotypic differences between cultivars in their field performance under drought. However, the reaction to drought is difficult to estimate in the field, being often confused with other factors, and only estimates based on yield data obtained during several years can be more or less reliable. Mustățea et al. (2009) estimated the response to drought in yield trials performed in 52 environments, which included a large variation of water availability, by calculating the intercept of the regression line of each cultivar yield versus average yield of the trial. Such field estimates of the drought response were available for 9 of the cultivars tested in this experiment as seedlings in the growth chambers.

We obtained a significant positive correlation between NDVI measured at day 23 in seedlings exposed to water stress and the intercept of regression line in 52 environmental field conditions (r=0.8*) (Table 3, Figure 7).

Table 3. Correlation between NDVI and yield response to drought in the field

	NDVI 23	NDVI 27
INTERCEPT	0.8*	0.4

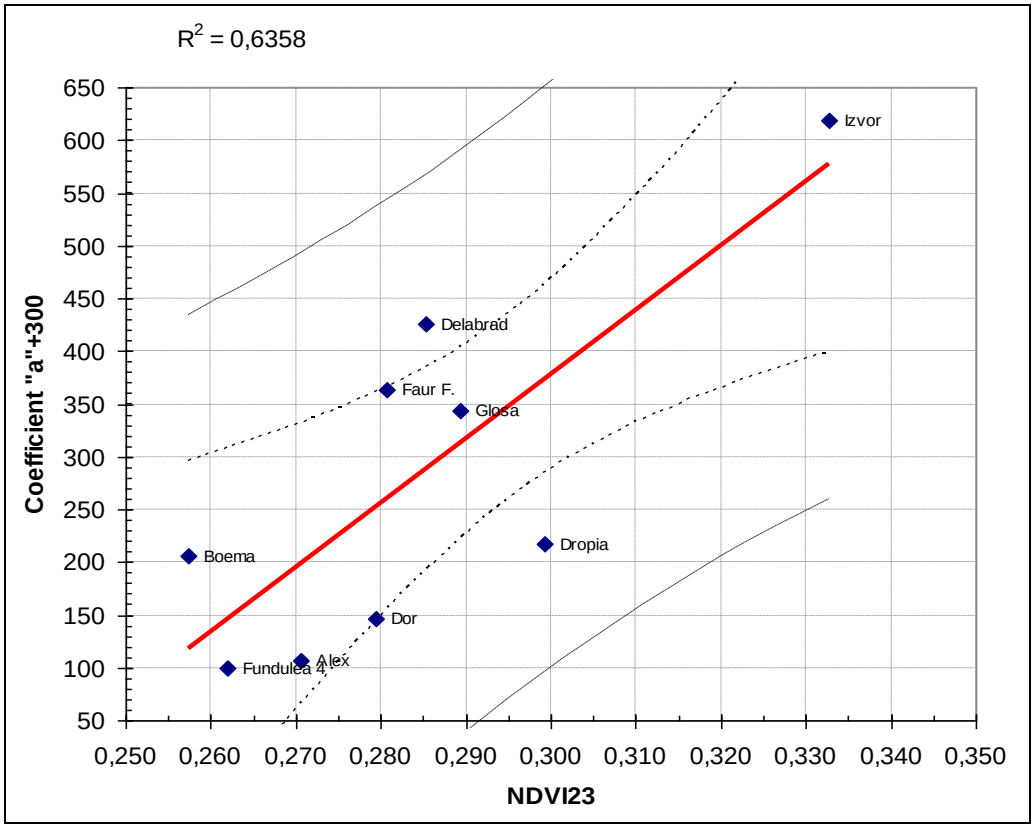

Figure 7. The relationship between NDVI and yield response to drought in the field

CONCLUSION

- We found significant variations between wheat genotypes for NDVI on day 23th of subjecting seedlings to water stress by gradual drying. We recommend carrying out analyses on the days when maximum differences between cultivars can be observed, which in our test were between 23th and 27th days.
- NDVI was correlated significantly with osmotic adjustment estimated by pollen test.
- Significant correlation was also found between NDVI and an approximate estimation of yield response to drought in the field.

• Measuring NDVI in seedlings exposed to water stress by gradual drying of the substrate can be used for testing a large number of genotypes, allowing a direct comparison between cultivars off-season, when field test cannot be performed. The obtained information could help enhancing genetic progress for osmotic adjustment and drought response.

Acknowledgements

I wish to thank the breeding and physiology teams that I worked with.

REFERENCES

Cutler, J.M., Steponkus, P.L., Watch M.J., and Shahan K.W., 1980. *Dynamic aspects and enhancement of leaf elongation in rice.* Plant Physiol., 66: 147-152.

David, M., 2012. *Pollen grain expression of intrisic and osmolyte induced osmotic adjustment in a set of wheat cultivars.* Romanian Agricultural Research, 29: 45-52.

David, M., 2013. *Seedling response to water stress induced by gradual drying of the substrate – a simple method to approximate drought resistance in wheat.* Romanian Agricultural Research, 30: 31-36.

Hackl, H., Mistele, B., Hu, Y., Schmidhalter, U., 2013. *Spectral assessment of wheat plants grown in pots and containers under saline conditions.* Functional Plant Biology, 40: 409-424.

Hsiao, T.C., 1973. *Plant responses to water stress.* Annu. Rev. Plant Physiol., 24: 519-570.

Hsiao, T.C., Acevedo, E., 1974. *Plant responses to water deficits, water use efficiency and drought resistance*. Agr. Met. 14: 59-84.

Lockhart, J.A., 1965. *Cell extension.* In: J. Bonner, J.E. Varner (eds.), Plant Biochemistry, Academic Press, New York: 827-849.

Morgan, J.M., 1988. *The use of coleoptile responses to water stress to differentiate wheat genotypes for osmoregulation, growth and yield.* Annals of Botany, 62: 193-198.

Mustățea, P., Săulescu, N.N., Ittu, G., Păunescu, G., Voinea, L., Stere, I., Mîrlogeanu, S., Constantinescu, E., Năstase, D., 2009. *Grain yield and yield stability of winter wheat cultivars in contrasting weather conditions*. Romanian Agricultural Research, 26:1-8.

Reynolds, M., Pask, A., Pietragalla, J., 2012. In: *Physiological Breeding II: A field guide to wheat phenotyping.* CIMMYT, Mexico: 10-15.

Saulescu, N.N., Kronstad, W.E., and Moss, D.N., 1995. *Detection of genotypic differences in early growth response to water stress in wheat using the Snow and Tingey system.* Crop Science, 35 (3): 928-931.