An automated image analysis system for seed vigour assessment in rapeseed

G. TOHIDLOO, M. KRUSE, H. DADGOSTAR, I. BORCHARDT, A.E. BAER and M. FRAUEN

Abstract

For vigour testing in rapeseed (*Brassica napus* L.), seeds were germinated in the dark under optimum conditions (no drought stress, 20 °C for 48 h) and under drought and temperature stress (PEG with water potential of -0.3 MPa at 30 °C for 48 h). Due to a vertical orientation of the blotters, seedlings were grown alongside the paper. For evaluation of seed vigour, blotters with seedlings were scanned with an inverted flatbed scanner and images were analysed with a self developed software program, called AutoVig. Mean and standard deviation of seedling area were determined. Differences between the seed lots were significant in both tests. Under optimum germination conditions seedling area was at least doubled compared to stress conditions. The results obtained with the AutoVig system from 34 rapeseed seed lots were correlated with field emergence data of the same seed lots from 20 field experiments. The mean field emergences ranged from 21 to 86%, indicating a broad stress variation. Significant positive correlations between AutoVig data obtained under stress and the field emergence percentages were found in 15 field experiments, between AutoVig data obtained under optimum conditions and the field emergence percentages in 19 field experiments. Moreover, the results of 19 field experiments were positively correlated with standard germination according to the ISTA Rules but correlation coefficients were generally lower than between field emergence and AutoVig.

Introduction

Many efforts have been done to employ computer based imaging systems to easily investigate different parts of plant or seed for different goals. Some of those systems simply consider seed size, shape and colour during the germination process under optimum and stress conditions (DELL'AQUILA 2007). MCCORMAC et al. (1990) developed an automated system for the assessment of vigour in lettuce (*Lactuca sativa* L.) seed lots.

By growing the seedlings using a slantboard test in which seeds were planted on a blotter and grown vertically in the dark, a video camera was able to capture gray-scale images of seedlings. But they were able to capture only five seedlings per image due to limitations imposed by the imaging device. Commercial seed vigour assessment systems also exist to determine seed vigour, such as the Ball Vision Index (CONRAD 1997) and the Paradigm System (MCNERTEY 1999). These systems capture and examine seedling parts such as cotyledon area (Ball Vision Index) or root length (Paradigm System). In general, such tests must be objective (even though subjective interpretation may be required), rapid (to meet client and laboratory requirements), simple (from a cost prospective and also to allow moderately trained personnel to conduct the test), economically practical (the costs involved should not outweigh the benefits), and reproducible (ISTA 1995).

Considering those expectations, in the last five years an automated image analysis system was developed by Sako and MCDONALD (2001) for vigour assessment of lettuce. The system addresses an imaging platform that can capture images of multiple seedlings from the side, enabling simultaneous measurements of both hypocotyls and radicles. Later on, they adjusted the system for cucumber (*Cucumis sativus* L.) and developed it for soybean (*Glycine max* L.), too (SAKO et al. 2004, MCDONALD et al. 2005). XU et al. (2005) tried to solve seedlings overlap in corn (*Zea mays* L.) which is one of the key notes of image analysis systems in species in which seedlings have multiple roots. HOFFMASTER et al. (2005) developed a seed vigour imaging system for three day-old soybean and corn seedlings which could classify the seedlings into six categories based on their shape. All above mentioned image systems were technically verified in the lab but not validated as vigour test by field emergence data obtained under stress conditions. Such a validation is required according to the ISTA seed vigour committee for the assessment of seed vigour tests. Therefore, the main objectives of this study were:

1. Development of an automated image analysis system for seed vigour assessment in rapeseed (*Brassica napus* L.), based on the principle of the system of Sako and MCDONALD (2001).
2. Validation of the system by comparison of the results with field emergence under different field conditions.

Material and Methods

For the experiments 34 seed lots from five different varieties of winter rapeseed (*Brassica napus* L.) were used, including seed lots with germination percentages below the certification threshold (85%).

In preparatory experiments it was found that germination in a water potential of -0.3 MPa at 30 °C for 48 h provides appropriate stress intensity for vigour testing in rapeseed. The water potential in this study was obtained with a polyethylene glycol (PEG) solution according to MICHEL and KAUFMANN (1973).

For vigour tests, 50 positions were marked on a blue blotter (Anchor Paper, St. Paul, MN, USA; landscape format

Autoren: MSc. Ghasem TOHIDLOO, Prof. Dr. Michael KRUSE, Universität Hohenheim, Institut für Pflanzenzüchtung, Saatgutforschung und Populationsgenetik, D-70599 STUTTGART; H. DADGOSTAR, Universität Stuttgart, Fakultät für Informatik, Elektro- und Informationstechnik, D-70569 STUTTGART; I. BORCHARDT, A.E. BAER and M. FRAUEN, Norddeutsche Pflanzenzucht Hans-Georg Lembke KG, D-24363 HOLTSEE

23×14 cm, 3 rows of 17, 16 and 17) by using a pencil and a template. The blotter was saturated with the PEG solution and placed inside a 25×21×2 cm plastic box (Bock, Lauterbach, Germany). Then, the seeds were planted at the positions and were covered with a wax paper sheet (23×14 cm, Anchor Paper, St. Paul, MN, USA) and another saturated blue blotter. On top of the blotter, the second replicate of 50 seeds was planted and so on. Each box finally comprised four replicates of 50 seeds and the top was covered with an extra blue blotter. The entire stack was carefully pressed with a flat plastic plate to fix the seeds at their positions. The plastic box was covered with a plastic foil and fixed firmly with a rubber band and placed upright, i.e. the blotter surfaces were at almost 90° from the horizontal, in a dark germinator at 30 °C for 48 h. After that period, the boxes were opened and the blotters with the seedlings were removed from the boxes and placed one by one into a rack that contains a flatbed scanner (hp-scan jet, model 4400 C) in an upside-down orientation in the cover (*Figure 1*). When closing the cover, the glass plate of the scanner is just above the seedlings on the blotter and images were scanned at high resolution (1200 dpi) and saved as JPG files.

For image analysis, a specific software program called AutoVig was written in Matlab 7.1 (The MathWorks, Inc. 3 Apple Hill Drive Natick, MA-US). The main procedure is the following: First, the blotter is extracted from the entire image. Then the colour of picture is converted to black and white and a grey threshold is applied to separate the bright seedlings from the dark background.

Afterwards, small non-seedling areas like non-germinated seeds, seed coats or reflections in the blotter were discarded by applying a minimum threshold for the pixel number of white areas. Then, the remaining white areas were counted and the number is shown on the program screen. Thereafter, the sizes of the white areas were determined in pixel and exported to an Excel file (Microsoft Excel, 2003). Also, mean size of the white areas and the standard deviation is indicated for the analyzed blotter on the screen.

For further statistical analyses, data were exported to Statistical Analysis System (SAS, version 9.13), e.g. for correlation analyses with field emergences.

For standard germination tests seeds were germinated in germination boxes on blue blotters according to the ISTA Rules (2005). Four replicates of 50 seeds were germinated at 20 °C for 7 days and the numbers of normal seedlings were recorded.

For field emergence tests, 20 field experiments were conducted in five different locations during 2005 and 2006. The experiments were done with different experimental designs, sowing depths and dates using the same 34 seed lots. It was taken care that no volunteer rapeseed was present. Field emergences were counted about two weeks after sowing when emergence was completed.

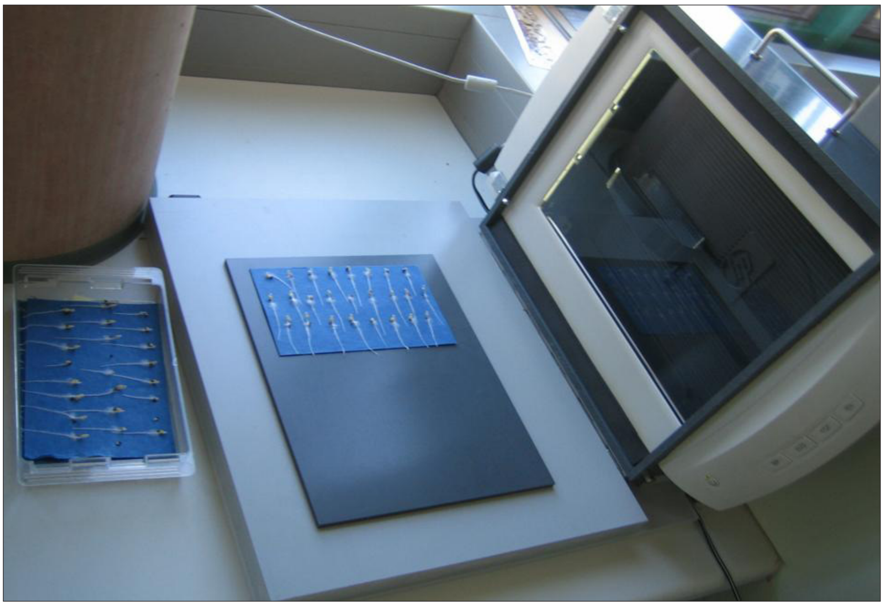

Figure 1: **The PVC box containing the upside-down orientated scanner in the cover**

Results

The image analysis system resulted average seedling sizes per seed lot between 326 and 472 pixels when germinated under stress conditions and between 630 and 1225 pixels under optimum conditions. *Table 1* shows that seedling sizes under stress conditions were considerably smaller than those under optimum conditions. The standard germination ranged between 76 and 99%.

Mean field emergences per field experiment ranged from nearly 20% (heavy stress condition) to 86% (optimum condition). Mean field emergences per seed lot ranged between 50 and nearly 65%.

Correlation coefficients between mean seedling size in the stress test, mean seedling size in the optimum test, percentage of normal seedlings in the standard germination test and field emergences in the 20 different experiments are shown in *table 2*. There were mostly positive correlations between the test results and field emergences. Out of 20 coefficients of correlation, 19 were significant for the correlation between seedling size in the optimum test and field emergence, 15 were significant for the correlation between seedling size under stress conditions and field emergence and 19 were significant for the correlation between standard germination and field emergence. Generally, most of the correlation coefficients between seedling size in the optimum test and field emergences were higher than those between standard germination and field emergence, which again were generally higher than those between seedling size in the stress test and field emergence.

Discussion

There are many laboratory and field emergence researches on different plant species which argue and proof that both germination velocity and uniformity play a decisive role for field emergence. These two properties are the most important and good predictors of seed vigour (SAKO and MCDONALD 2001).

Image analysis is a very fast and objective tool for measuring seedling size in

Table 1: **Mean seedling area of the seed lots germinated under optimum (SAVN) and stress conditions (SAPE)**

Seed lot	SAVN (Pixel)	SAPE (Pixel)	Seed lot	SAVN (Pixel)	SAPE (Pixel)
1	630	459	18	890	457
2	671	337	19	895	441
3	714	456	20	896	434
4	725	374	21	902	447
5	740	328	22	904	441
6	745	348	23	908	443
7	790	326	24	940	409
8	790	373	25	955	428
9	806	428	26	993	431
10	808	352	27	1000	416
11	810	356	28	1001	460
12	817	356	29	1022	421
13	825	376	30	1105	456
14	858	472	31	1112	426
15	860	438	32	1139	447
16	878	367	33	1161	427
17	880	424	34	1225	433

seed vigour testing. Both, germination velocity and uniformity can be obtained easily within a short period of time. The meaning of the measured seedling size values as estimates for seed vigour mainly depends on the kind of germination test, the germination conditions, the measured characteristic and finally also on the precision and accuracy of the measuring tool. As a consequence, developed image analyses systems for seed vigour evaluation for a certain species may not work for others. For example rapeseed has a short hypocotyl in comparison with lettuce so that a system adapted to lettuce does not function for rapeseed. AutoVig is specially designed and developed for image analysis of rapeseed. The germination method is done in a kind of „sandwich"-arrangement of the 4 replicates of 50 seeds. Germination is done in a box placed in an incubator at 90° from horizontal for 48 h only. There is a possibility to ignore dead seed and remove small image noises in AutoVig as well. This gives a chance to the user to consider dormant seeds in a lot. Removing small noises will decrease standard deviation of seedlings within replications of a lot and with it increase accuracy of the system. Nonetheless, no small seedling should be removed from the image, since removing of the small seedling might have an influence on the judgment of seed vigour of seed lots. Therefore, it is essential to have a high quality-standardized image analysis system for seed vigour assessment for different species. Another difference of AutoVig compared with other software is the possibility to export the related data of each seed lot into an excel table and observe directly the mean and standard deviation between seedlings and further statistics could be carried out. Thus, AutoVig is a reliable system since the images are objective and all data are accessible for a detail controlling. Moreover, the system is speedy, reproducible and economical. Although this method is developed for rapeseed, it may applicable for other species with the same germination characteristics as well. Finally, the results obtained by AutoVig from germination under optimum and stress conditions have proved its close relation to field emergences under various conditions. Other systems were not validated by field emergence tests. This validation is particularly expressed by the ISTA Vigour Test Committee for verification of every vigour test.

Table 2: **Correlations between seedling area under stress conditions (SAPE), under optimum conditions (SAVN) and standard germination percentage (NGer) with field emergences in 20 different field experiments**

field experiment	SAPE	SAVN	NGer	field experiment	SAPE	SAVN	NGer
BD0512	0.35*	0.82*	0.52*	HD064	0.46*	0.71*	0.47*
BD0514	0.42*	0.64*	0.42*	HD066	0.27ns	0.45*	0.33*
BD0522	0.53*	0.54*	0.56*	HL051	0.43*	0.58*	0.54*
BD0524	0.62*	0.54*	0.57*	HL052	0.48*	0.62*	0.57*
BD062	0.26ns	0.48*	0.33*	HL06	0.60*	0.64*	0.63*
BD064	0.08ns	0.50*	0.32*	MA051	0.51*	0.46*	0.62*
BD066	0.26ns	0.40*	0.55*	MA052	0.64*	0.46*	0.76*
HD051	-0.23ns	0.16ns	-0.04ns	MA06	0.36*	0.55*	0.48*
HD052	0.52*	0.50*	0.52*	OL051	0.35*	0.59*	0.42*
HD062	0.43*	0.60*	0.51*	OL052	0.44*	0.65*	0.43*

BD0512: Bad Lauchstädt, sowing date 1, sowing depth 2 cm, 2005
HD064: Heidfeldhof, sowing depth 4 cm, 2006
BD0514: Bad Lauchstädt, sowing date 1, sowing depth 4 cm, 2005
HD066: Heidfeldhof, sowing depth 6 cm, 2006
BD0522: Bad Lauchstädt, sowing date 2, sowing depth 2 cm, 2005
HL051: Hohenlieth, sowing date 1, 2005
BD0524: Bad Lauchstädt, sowing date 2, sowing depth 4 cm, 2005
HL052: Hohenlieth, sowing date 2, 2005
BD062: Bad Lauchstädt, sowing depth 2 cm, 2006
HL06: Hohenlieth, 2006
BD064: Bad Lauchstädt, sowing depth 4 cm, 2006
MA051: Malchow, sowing date 1, 2005
BD066: Bad Lauchstädt, sowing depth 6 cm, 2006
MA052: Malchow, sowing date 2, 2005
HD051: Heidfeldhof, sowing date 1, 2005
MA06: Malchow, 2006
HD052: Heidfeldhof, sowing date 2, 2005
OL051: Oberer Lindenhof, sowing date 1, 2005
HD062: Heidfeldhof, sowing depth 2 cm, 2006
OL052: Oberer Lindenhof, sowing date 2, 2005

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