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Received: 2018-07-13 ; Accepted: 2018-08-31

CHLOROPHYLL FLUORESCENCE AS A QUALITY INDICATOR OF ROOT PARSLEY (*Petroselinum crispum* var. Lenka) SEEDS

Summary

Requirements concerning seed quality are growing constantly. Parsley is a plant species producing seeds with low genetic seed vigour. Parsley seeds are usually germinating slowly and this process is extended in time. Lack of uniformity in field emergence results in a lower root yield and quality. Plants emerging on different days produce roots of different size and weight. Improvement of parsley seed quality is an important issue. A selection of seeds according to their quality can be one of the methods. Chlorophyll fluorescence measurement is a quick, non-destructive method and its results are negatively correlated to the germination ability and field emergence.
***Key words**: root parsley, seed vigour, seed quality, chlorophyll fluorescence*

FLUORESCENCJA CHLOROFILU JAKO WSKAŹNIK JAKOŚCI NASION PIETRUSZKI KORZENIOWEJ (*Petroselinum crispum*) ODMIANY LENKA

Streszczenie

Wymagania dotyczące jakości materiału siewnego stale rosną. Pietruszka zwyczajna należy do gatunków tworzących nasiona o niskim wigorze genetycznym. Nasiona pietruszki zazwyczaj kiełkują powoli, a proces kiełkowania jest rozciągnięty w czasie. Nierównomierne wschody polowe w konsekwencji powodują obniżenie plonu i jakości korzeni. Rośliny, które wzeszły w innym czasie wytwarzają korzenie zróżnicowane pod względem wielkości i masy. Poprawa jakości nasion pietruszki jest więc istotnym zagadnieniem. Jedną ze stosowanych metod może być selekcja nasion o najwyższej jakości. Pomiar fluorescencji chlorofilu jest metodą szybką i niedestrukcyjną, a poziom fluorescencji jest ujemnie skorelowany ze zdolnością kiełkowania i wschodami polowymi.
***Słowa kluczowe**: pietruszka korzeniowa, wigor nasion, jakość nasion, fluorescencja chlorofilu*

1. Introduction

Modern agriculture requires sowing material of high quality. Field emergence is not always correlated to laboratory germination ability but it is crucial for plant uniformity and quality during the harvest. Therefore high mechanized modern plant production demands seeds of excellent quality. Most of seed vigour tests are destructive and seeds after the examination are not suitable to sow. Chlorophyll fluorescence measurement is a quick and non-destructive method of seed evaluation. Examined seeds can be sown under laboratory or field conditions right after the assessment of their quality.

Measurement of chlorophyll fluorescence is simple, fast and the most important - non-destructive [10]. Chlorophyll fluorescence can be defined as the red to far-red light emitted by photosynthetic tissues/organisms when illuminated by light of approximately 400–700 nm (photosynthetically active radiation or PAR). Although chlorophyll fluorescence represents only approximately 0.5–10% of the absorbed energy, its intensity is inversely proportional to the fraction of energy used for photosynthesis (a redox effect).For this reason, the chlorophyll fluorescence signal can be used as a probe for photosynthetic activity [11]. Value of chlorophyll fluorescence can be used as a tool for examination of photosynthetic reactions for stress conditions and evaluation of an influence of unfavorable environmental conditions on plants [12]. Chlorophyll fluorescence is one of the physiological parameters that can indicate cold tolerance [1] vitality of plants (trees) [9], or reaction to shortage of minerals [17].

Chlorophyll fluorescence measurements are used for example in:

- agriculture [10, 14],
- horticulture [3],
- forestry [13, 16],
- seed science [6, 7, 8].

Chlorophyll fluorescence is strictly connected with photosynthesis. Chlorophyll is present in unripe seeds. During seed ripening process chlorophyll degrades and its content and activity decreases. Mature seeds don't show presence of photosynthetic active chlorophyll. Therefore seeds chosen based on the lowest values of chlorophyll fluorescence have usually the highest vigour and the highest quality seeds.

Root parsley is a species whose seeds show high differences between laboratory germination ability and field emergence. Methods of parsley seed examinations still need improvement in order to find ones better correlated to the field emergence. Chlorophyll fluorescence measurement can be one of these methods.

2. Materials and methods

Seeds of root parsley variety Lenka were the object of experiment. This variety is described as medium late with aromatic flesh and good storage value. Parsley variety Lenka requires fertile soil with good water conditions.

Chlorophyll fluorescence level was detected with Impulsive Fluorimeter Handy Pea made by Hansatech Instruments (Fig. 1).

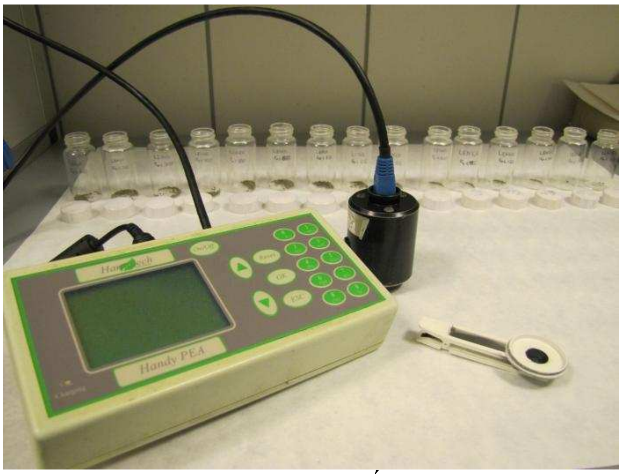

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Fig. 1. Fluorimeter Handy Pea
Rys. 1. Fluorymetr Handy Pea

Seeds were divided into three groups based on different values of chlorophyll:
- I group F_0 0-200,
- II group F_0 400-500,
- III group $F_0 > 700$.

F_0 is a chlorophyll fluorescence intensity obtained at 50 μs.

As a control combination the unseparated seeds from the same variety and seed lot were used.

In the laboratory for each seed group: laboratory germination ability, mean time of single seed germination (Pieper`s coefficient) and germination uniformity were determined.

Seeds germinated in Petri`s scales on the blotting-paper rings soaked with distilled water at light presence, in the temperature of 21°C. Each combination (I, II, III and control) consisted of 50 seeds in 3 replications. Germination was measured for 14 days. Each day germinated seeds were counted and removed from the scale. After that laboratory germination ability, mean time of germination (measured with Pieper`s coefficient) and germination uniformity were counted for each seeds group.

Fields experiments were conducted in Mierzeniec near Pułtusk in masovian district. Seeds of root parsley variety Lenka were separated based on the same values of chlorophyll fluorescence as in the case of laboratory experiments. Seeds of each group were sown on 30 of March 2014 in 3 replications, 50 seeds each. Seeds after sowing and emerging seedlings were growing in natural field conditions. Soil was sowing IIIb class with winter rape used as a forecrop. After rape crop full set of agriculture operations were made. During the vegetation period hand weeding was the only agrotechnical treatment.

Weather during the field experiment was quite wet, rainfall was higher than mean value, except of June and September (Table 1). Especially April, May and August were rainy what affected parsley growth.

For each seed combination: field emergence rate, emergence speed and uniformity, chlorophyll contents in leaves and leaf area index (LAI) were determined. During the harvest the total yield, root yield and number of plants were measured. Because of low number of plants obtained from 150 seeds, plants from every combination were counted and weighed together, with no replications. Harvest of root parsley was done on 25th September 2014.

Chlorophyll content was measured with Chlorophyll Meter Minolta Spad 502 – chlorophyll content in leaves is expressed in contract units (Fig. 2).

Leaf Area Index was measured with LAI_2000 equipment used to measurement of canopy light absorption (Fig. 3).

Table 1. Weather conditions during field experiment.
Tab. 1. Warunki pogodowe podczas doświadczeń polowych

	Rainfall [mm]		Temperature [°C]	
	2014	multi-annual average	2014	multi-annual average
March	28,0	27,0	6,4	2,1
April	49,8	38,7	10,3	7,8
May	92,6	55,2	14,1	13,6
June	60,2	63,5	16,4	16,7
July	82,8	80,7	20,9	18,6
August	81,1	67,7	17,9	17,6
September	32,7	46,4	14,4	13,4

Source: own work / Źródło: opracowanie własne

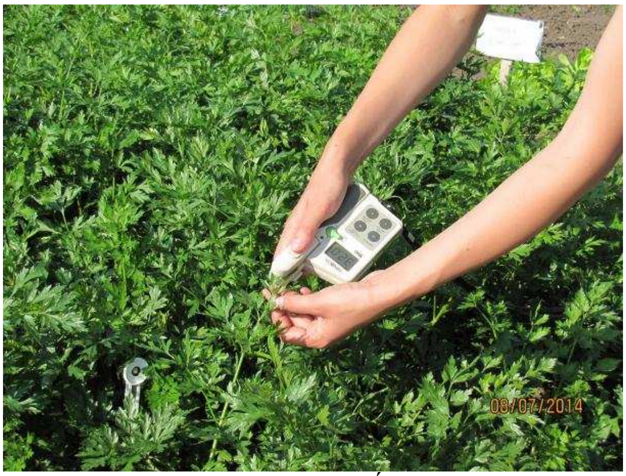

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Fig. 2. Measurement of chlorophyll level of parsley leaves with Chlorophyll Meter Minolta Spad 502
Rys. 2. Pomiar zawartości chlorofilu w liściach pietruszki za pomocą chlorofilometru Minolta Spad 502

Source: photo M. Stypińska / Źródło: fot. M. Stypińska

Fig. 3. Measurement of parsley canopy with LAI-2000
Rys. 3. Pomiar łanu pietruszki za pomocą aparatu LAI-2000

3. Results
3.1. Laboratory experiments

Results of laboratory germination (Table 2) show that seed quality was negatively correlated to the level of chlorophyll fluorescence. Seeds selected on the basis of the lowest chlorophyll fluorescence performed best. They germinated in more than 70%, while those of highest fluorescence level only in 30%. They germinated fast, earlier by almost 1,28 day than seeds which showed the highest level of chlorophyll fluorescence. Their germination was the most uniform. There were no significant differences in germination between seeds with low fluorescence and control ones.

Table 2. Laboratory germination of parsley seeds depending on chlorophyll fluorescence level.
Tab. 2. Przebieg kiełkowania nasion pietruszki w zależności od poziomu fluorescencji chlorofilu

Laboratory germination ablility (%)				
Control	F_0<200	F_0 400-500	F_0>700	LSD
58,0b	70,7b	58,0b	30,7a	17,12
Mean germination time (days)				
Control	F_0<200	F_0 400-500	F_0>700	LSD
8,74a	8,52a	8,68a	9,80b	0,77
Germination uniformity (days)				
Control	F_0<200	F_0 400-500	F_0>700	LSD
2,74a	2,52a	2,68a	3,80b	0,77

Source: own work / Źródło: opracowanie własne

3.2. Field experiments

Similarly in the field (Table 3) seeds chosen on basis of lowest chlorophyll fluorescence gave the highest field emergence rate (63%). Seedlings from these seeds emerged quicker and more evenly than from the seeds showing high chlorophyll fluorescence.

Table 3. Field emergence of parsley depending on seed chlorophyll fluorescence level
Tab. 3. Wschody polowe pietruszki w zależności od poziomu fluorescencji chlorofilu nasion

Field emergence rate (%)				
Control	F_0<200	F_0 400-500	F_0>700	LSD
57,3c	63,3c	38,0b	14,7a	12,49
Mean time of emergence (days)				
Control	F_0<200	F_0 400-500	F_0>700	LSD
22,0a	22,1a	23,0ab	23,9b	1,02
Uniformity of emergence (days)				
Control	F_0<200	F_0 400-500	F_0>700	LSD
2,95a	3,10a	4,00ab	4,90b	1,02

Source: own work / Źródło: opracowanie własne

Chlorophyll content in leaves of examined plants during the vegetation period is presented in Table 4.

Chlorophyll content in leaves is of crucial importance for plant productivity. During most of measurements plants obtained from the seeds with high chlorophyll level showed significantly lower chlorophyll content in leaves than other combinations. Parsley plants from seeds, whose chlorophyll fluorescence was lower, were more vital and more resistant to stresses.

Leaf Area Index (LAI) of parsley plant from different types of seeds is presented in Table 5.

Table 4. Chlorophyll content in leaves of parsley during the vegetation period
Tab. 4. Zawartość chlorofilu w liściach pietruszki podczas okresu wegetacji

Combination	**Date of measurement**			
	08.07	**28.07**	**27.08**	**25.09**
Control	34,57bAB	38,32bB	30,24bA	34,13bcAB
Fo < 200	33,02abA	43,03cB	35,71cA	40,74cB
Fo 400-500	35,80bB	44,5cC	29,46abA	32,24bAB
Fo >700	28,93aB	29,43aB	26,81aAb	24,20aA
LSD	5,49	3,29	3,15	6,79

a, b, c - homogeneous groups for different combinations
A, B, C - homogeneous groups for date of measurement
Source: own work / Źródło: opracowanie własne

Table 5. Leaf Area Index (LAI) of examined parsley plants
Tab. 5. Wskaźnik powierzchni liści (LAI) badanych roślin pietruszki

Combination	**Date of measurement**			LSD
	25.07	**28.07**	**27.08**	
Control	4,54bB	3,21bcA	4,96bcB	1,24
Fo < 200	7,38cC	4,07cA	5,70cB	0,92
Fo 400-500	4,65bB	2,72abA	4,51bB	0,60
Fo > 700	2,76aA	2,20aA	2,58aA	0,59
LSD	0,77	0,58	1,06	

a, b, c - homogeneous groups for different combinations
A, B, C - homogeneous groups for date of measurement
Source: own work / Źródło: opracowanie własne

Leaf area, similarly as a chlorophyll content, strongly influences plant yield and its quality. The highest value of LAI was measured for plants grown from seeds Fo <200 and the for plants grown from seeds Fo>700. Parsley plants from the seeds with the lowest chlorophyll fluorescence had leaves area 60% larger than control ones. Plants from the seeds with the highest chlorophyll fluorescence produced leaf area almost 50% smaller than control. All differences between plant groups were significant.

During the harvest plant number, weight and root weight were measured (Table 6). All the plants from one combination were picked together, so there was no possibility to analyze the results statistically.

Table 6**.** Total yield, root yield and number of plants
Tab. 6. Plon całkowity, plon korzeni i liczba roślin

	Total yield (g)	Root yield (g)	Number of plants
Control	3480	1680	86
Fo<200	3470	1810	95
Fo 400-500	2740	1470	57
Fo > 700	2220	1310	22

Source: own work / Źródło: opracowanie własne

It is visible that total yield and root yield were higher in case of plants obtained from seeds with lower chlorophyll fluorescence and control ones, comparing to the seeds with higher levels of chlorophyll fluorescence.

4. Discussion

Chlorophyll fluorescence is an interesting physiological parameter which can be used for many purposes. Generally it is used for describing current plant condition especially subject to abiotic stresses like drought, frost, soil salinity, shortage of some elements etc. It can be also used as an indicator of fruit maturity, in monitoring of water ecosystems, for detection of biological threats and in seed vigour testing [2].

Root parsley is a difficult crop because of extended in time and irregular field emergence. Therefore finding a proper seeds vigour tests for parsley seeds is an important issue. Among many vigour tests chlorophyll fluorescence measurement is the only non-destructive method, remaining examined seeds in the same morphological and physiological condition. As a non-destructive method it can be used as an indicator of seed material quality of such species as: cabbage, pepper, tomato, fennel, grasses, tobacco, rice, carrot, cotton, sunflower and beet [15].

Jalink et al in 1998 [5, 6] for seeds of cabbage and in 1999 [7] for tomato seeds obtained results very similar to these presented for parsley in this paper. They confirmed the reverse dependence of seed vigour and their chlorophyll fluorescence [5, 6, 7]. White cabbage and tomato seeds separated according to a low chlorophyll fluorescence level germinated faster and more uniformly than seeds with high chlorophyll fluorescence level. It is possible to divide one, seemingly uniform batch of seeds for portions clearly differing in color and consequently in their quality too [8].

From 2003 to 2006 Department of Plant Physiology Warsaw Agricultural University examined dependence of seeds vigour on chlorophyll fluorescence for carrot, wheat, sugar beet, rape and triticale seeds [4]. Results of these experiments confirmed that lower level of chlorophyll fluorescence indicated higher seeds vigour, but not for all species. However this method of seed examination is not recommended for all the crop species. For example for rape the differences were too small (only 10-20 units) between high and low vigorous seeds.

5. Conclusions

1. Chlorophyll fluorescence of parsley seeds is negatively correlated to their quality measured by germination ability, speed and uniformity.
2. Plants from seeds selected based on low chlorophyll fluorescence emerge in the field in a large percentage, quickly and evenly.
3. Plants from seeds with a low chlorophyll fluorescence value develop larger canopy, their leaves contain more chlorophyll and in consequence give higher yield than other seed combinations, especially under unfavorable environmental conditions.
4. Chlorophyll fluorescence level is suitable indicator of seed quality and maturity for root parsley.

6. References

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Own Research Fund / Badania własne