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IDENTIFICATION OF NEW POLISH LINES OF *Chenopodium quinoa* (Willd.) BY SPECTRAL ANALYSIS OF PIGMENTS AND A CONFIRMATION OF GENETIC STABILITY WITH SCoT AND RAPD MARKERS

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ABSTRACT

Identification of cultivars is essential both in breeding and to settle cultivar disputes. The purpose of the study has been to examine cultivar identities based on absorption spectra of plant pigments and to confirm a genetic stability with SCoT and RAPD molecular markers in new Polish lines of *Chenopodium quinoa* Willd. Spectral analysis of pigments extracted from plant inflorescences in quinoa gives an opportunity to confirm the cultivar identity and identification of 'Faro' and 'Titicaca' cultivars and their new lines. Spectral analysis is an effective method of confirming cultivar identity and it should be used in practice for the identification of cultivars or cultivars lines in *Chenopodium quinoa* Willd. Analysis of molecular markers indicated by RAPD as well as SCoT technique revealed a high genetic stability of the derivative lines of 'Faro' and 'Titicaca', while variation was detected in plants representing original cultivars: banding pattern different than predominant was present in three plants of 'Titicaca' (genetic distnaces from 7.5% to 55.9%) and in a single plant of 'Faro'(genetic distance 61.2% as indicated by SCoT technique).

Key words: breeding, molecular markers, quinoa, pigments

INTRODUCTION

Chenopodium quinoa Willd. is a species of the *Amaranthaceae* family (from phylogenetic system APGIII) formerly called *Chenopodiaceae* (in the Reveal classification system). Due to a different systematic affiliation and a wide use of its seeds in nutrition, it is referred to as pseudocereals. Quinoa comes from South America from Ecuador, Peru and Bolivia. It has been cultivated at least since the beginning of the era by American Indians and has since been brought to Argentina and Chile. It has also been a fundamental component of diets in these countries since ancient times [Zañudo 2015]. Its advantage, in addition to low soil and climatic requirements, is its high competitiveness with most weeds [Martínez et al. 2009]. It is characterized by great tolerance to cold and frost to –5°C. It is resistant to long-term drought. Quinoa also offers a high nutritional value, which surpasses commonly cultivated species [Ruiz and Bertero 2008, Gęsiński 2009, Hirose et al. 2010].

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It is one of few plant products with the right proportions of protein, carbohydrates and minerals indispansible for human life [Meyhuay 2014]. It provides carbohydrates and significant amounts of minerals, especially phosphorus, potassium and magnesium, but also calcium, sodium, iron (50% more than in cereals), copper, manganese and zinc [Jacobsen 2015]. Quinoa seeds also contain 58 to 68% of starch and 5% of sugar [Meyhuay 2014]. However, the biological value of the plant results primarily from the quality of protein (amino acid composition) [Cauda et al. 2013]; an ideal balance of eight essential exogenous amino acids, especially lysine and tryptophan [Ruas et al. 1999]. Another important factor of the biological value of this species is protein digestibility, which accounts for about 80%. The seeds of this species contain 78.0 $g \cdot kg^{-1}$ of total dietary fiber. Quinoa is also a good source of vitamins B: riboflavin and folic acid, it also contains thiamine, and significant amounts of vitamin E (tocopherols) and vitamin C [Cauda et al. 2013]. The plant is also rich in high quality lipids, polyunsaturated fatty acids (omega 3 and omega 6) [Cauda et al. 2013], as well as saponins and, as such, the plant can be applied for biomedical purposes [Jancurová et al. 2009, Vega-Gálvez et al. 2010]. The combination of factors contributes to excellent antioxidant properties. The chemical study of the seeds also revealed the presence of a number of polyphenols, dominated by kaempferol and quercetin glycosides [Dini et al. 2004].

Pigments are responsible for the plant colour found in organelles (vacuoles, chloroplasts or chromoplasts). The main pigments in plants are carotenoids (yellow, orange, red), flavonoids (cream or light yellow flavones and flavonols and blue, violet or red anthocyanins) and found in the family *Amaranthaceae* betalains (red-violet betacyanins and yellow-orange betaxantins) [Harborne 1988, Takeda et al. 1994, Strack et al. 2003, Gonçalves et al. 2012, Des Marais 2015]. However, it turns out that the plants producing anthocyanins will not synthesize betalains (Stafford, 1994). Differences in the colour of the plant organs (flowers, leaves, roots) are a springboard for distinguishin the species of new cultivars. In the quinoa breeding program in the Netherlands, Center for the Plant Breeding and Reproduction Research – Agriculture Research Department, inflorescence colour is used to identify successful hybrids of parents which differ in inflorescence colour [Peterson et al. 2015]. In the world, hundreds of new plant cultivars are produced each year, which triggers more and more problems in identifying them. With that in mind, a quick and inexpensive method is needed to distinguish even very similar cultivars. Kawase and Tsukamoto [1974, 1976] carried out spectral analysis in intact florets. The study of the content of pigments in inflorescences giving a basis for demonstrating differences across chrysanthemum cultivars was performed by Lema-Rumińska and Zalewska [2004, 2005].

Molecular markers are commonly used both to confirm the genetic stability and to detect variation between plants, even in a closely related group of plants. The Random Amplified Polymorphic DNA marker (RAPD), first proposed by Welsh and McClelland [1990] and Williams et al. [1990] is often applied in the studies of genetic identity as well as variation of crops and it was used, side by side with AFLP and SSR markers, by Maughan et al. [2004] to create the genetic linkage map of quinoa. The Start Codon Targeted Polymorphism (SCoT) molecular markers technique was proposed by Collard and Mackill [2009] and has not been yet applied for quinoa examination. SCoT markers has recently gained much attention since this method is technically as easy as RAPD, however, without its poor reproducibility. Single primers used for SCoT markers are usually 18-mers based on the ATG-start codon conserved region, typical PCR is conducted and the annealing temperature about 50°C .

The purpose of the study has been to examine the cultivar identities based on morphological characteristics and absorption spectra of plant pigments and to confirm the genetic stability with molecular SCoT and RAPD markers in *Chenopodium quinoa* Willd. plants, 'Titicaca' and 'Faro' cultivars and their new Polish lines: 'Titicaca White', 'Titicaca Red' and 'Faro White', 'Faro Orange', 'Faro Red'.

MATERIAL AND METHODS

The plant material involved two original quinoa cultivars: 'Faro', a cultivar from Argentina and 'Titicaca', a cultivar from Denmark. For several years of

introducing the material to Polish conditions, a single individual which differed in morphological characteristics and in particular staining of inflorescences and faster flowering from the original cultivars was observed. The isolated material was propagated by evaluating the alignment and sustainability of the characteristics for the next five years; the characteristics were repetitive. From the two original cultivars Krzysztof Gęsiński selected the following lines: 'Faro White', 'Faro Orange', 'Faro Red' and 'Titicaca White' and 'Titicaca Red' (fig. 1).

Analysis of morphological traits and pigments

For all the cultivars lines the seed was sown on March 9, 2016 to a mixture of peat and perlite in the 2 : 1 ratio to multiples (20 seeds of two cultivars of quinoa origin: 'Faro' and 'Titicaca' and their 5 new lines). After three weeks of sowing, the plants were transplanted into 29 cm diameter pots (5 plants/ pot) and grown in a greenhouse under controlled temperature and lighting conditions until full flowering.

During full flowering of plants, selected morphological features were measured: shoot length, length and width of inflorescences, and the number of leaves. Results were statistically verified with variance analysis and the Fisher test (Statistica'12, StatSoft, Poland), at significance level $\alpha = 0.05$.

The inflorescence colour was also assessed using the RHSCC chart [The Royal Horticultural Society Colour Chart 1966], under the same light conditions.

From fresh inflorescences, with a colour characteristic for quinoa cultivar/ cultivar line 10 samples of 100 mg each were prepared for extraction of carotenoids and flavones and flavonoids and 10 samples of 200 mg for the extraction of anthocyanins.

The samples were homogenized by rubbing in a porcelain mortar with several mg quartz sand according to the procedure described by Lema-Rumińska and Zalewska [2004]. Carotenoids, flavones and flavonoids pigments were extracted with 100% acetone from and anthocyanins with 1% HCl of methanol. The resulting extracts were filtered to a volume of 10 ml through a filter paper. The absorbance was measured using a UV-VIS 1601 spectrophotometer in 1 cm-wide quartz cuvettes (Shimadzu, Japan). After the readings were made, the absorbance maxima and the average absorption coefficients for the individual wavelengths were determined. All the values were expressed as mean ± standard deviation (SD) of completely independent replications.

Molecular markers analysis

To evaluate the genetic stability of original as well as derivative cultivars, an analysis was performed using a RAPD technique [Welsh and McClelland 1990, Williams et al. 1990] and SCoT molecular markers [Collard and Mackill 2009]. For analysis, nine primers (DNA-Gdansk, Poland) were selected for RAPD technique as well as nine primers were used for SCoT technique. The names and sequences of primers used are provided in detail in Table 1.

From each of the two original cultivars ('Faro' and 'Titicaca') as well as from each of the five derivative lines, ten field cultivated plants were selected for a DNA extraction. Genomic DNA was extracted from 100 mg of young and fresh leaves using a Genomic Mini AX Spin Plant Kit (A&A Biotechnology, Poland), according to the manufacturer's instructions. The quality and concentration of extracted genomic DNA were estimated using Quantus fluorometer (Promega, USA) and the final concentration of DNA templates was set to 20 ng/µl.

The DNA was amplified in 25 µl reaction volumes, containing 20 ng of genomic template DNA, 12.5 µl of PCR Mix Plus (containing *Taq* RUN polymerase, $MgSO_4$ and a set of dNTPs, manufactured by A&A Biotechnology, Poland) and the final concentration 1 µM of a single primer. The amplification was performed under the following conditions: RAPD – initial denaturation for 4 min at 94°C; followed by 35 cycles of 94°C for 1 min, 36°C for 1 min, 72°C for 2 min; final extension of 72°C for 4 min; SCoT – initial denaturation for 4 min at 94°C; 35 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 2 min; final extension of 72°C was held for 10 min, after the PCR samples were kept at 4°C. The amplification products were separated using 1.5% (w/v) agarose gel electrophoresis, stained with ethidium bromide, in TBE buffer at 120 V. DNA GeneRuler Express DNA Ladder (Thermo Fisher Scientific, USA) and Genoplast 5000bp (Genoplast, Poland) were used to determine the fragments size. The bands were visualized using the GelDoc system (BioRad, USA).

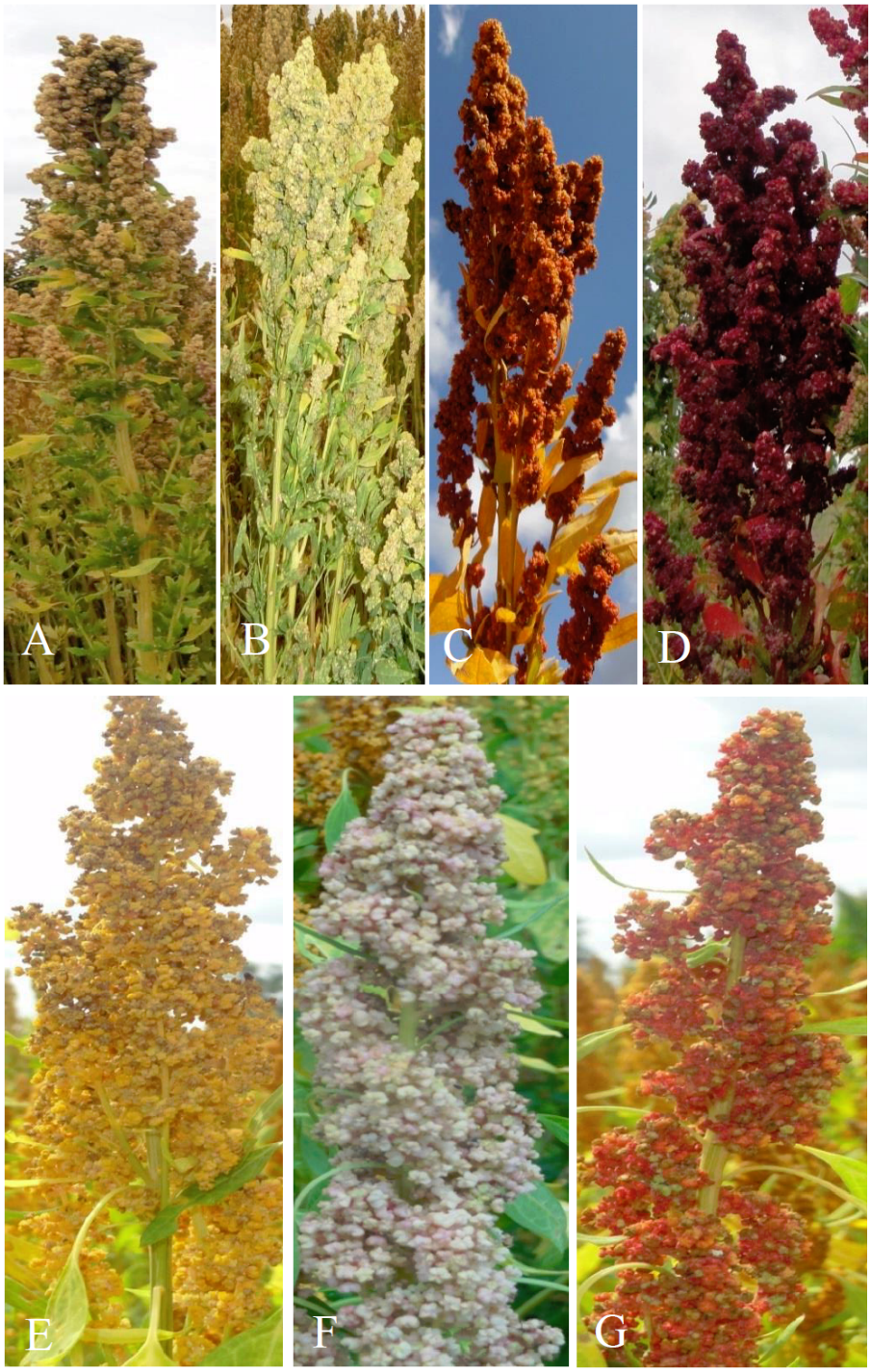

Fig. 1. Cultivar 'Faro' and 'Titicaca' and their lines: A – 'Faro' original, B – 'Faro White', C – 'Faro Orange', D – 'Faro Red', E – 'Titicaca' original, F – 'Titicaca White', G – 'Titicaca Red'

Table 1. Names and sequences of primers used in the analysis of genetic stability of 'Faro' and 'Titicaca' quinoa cultivar groups

RAPD primers		SCoT primers	
Name	5'–3' sequence	Name	5'–3' sequence
A	GGG AAT TCG G	SCoT 3	CAA CAA TGG CTA CCA CCG
B	GAC CGC TTG T	SCoT 4	CAA CAA TGG CTA CCA CCT
C	GGA CTG GAG T	SCoT 8	CAA CAA TGG CTA CCA CGT
D	GCT GCC TCA GG	SCoT 12	ACG ACA TGG CGA CCA ACG
E	TAC CCA GGA GCG	SCoT 13	ACG ACA TGG CGA CCA TCG
F	CAA TCG CCG T	SCoT 25	ACC ATG GCT ACC ACC GTC
G	GGT GAC GCA G	SCoT 26	ACC ATG GCT ACC ACC GTC
I	TGG CGT CCT T	SCoT 27	ACC ATG GCT ACC ACC GTG
J	AGC GTG TCT G	SCoT 33	CCA TGG CTA CCA CCG CAG

All RAPD and SCoT loci, indicated for each genotype, were counted using a binary scoring system recording the presence or absence of bands as 1 and 0, respectively. The genetic distances (%) were estimated according to Nei and Li [1979] using Treecon v. 1.3 program [van de Peer and de Watcher 1994].

RESULTS

Morphological characteristics

The 'Faro' group demonstrated a significantly higher shoot length than the 'Titicaca' group. The highest shoot length was found in 'Faro Red', and the lowest – in 'Titicaca' original and 'Titicaca Red' (tab. 2). Also the number of leaves varied across quinoa cultivars. 'Faro' cultivars showed a significantly higher number of leaves than the cultivars representing the 'Titicaca' group. The highest number of leaves occurred in the original 'Faro' cultivar, while the lowest number of leaves – in 'Titicaca' cultivars: 'Titicaca' original and 'Titicaca Red'. The 'Faro Red' cultivar line demonstrated a significantly longer length and broader inflorescence as compared to the other cultivars and lines, except for 'Faro White'.

The cultivars differed in inflorescence colour (tab. 2). The most distinctive of the original cultivars was 'Faro Orange', classified as a colour group, according to the RHSCC chart, as Orange-Red colour code 34/ 145B and 'Titicaca White' as the Yellow group, colour code 2C. The most similar in terms of inflorescence colour were the cultivars of two different cultivar groups: 'Faro Red' and 'Titicaca Red'. Both cultivars were characterized by red inflorescences: Red-Purple 65A or 65B (depending on the cultivar) and the dominant colour group Yellow-Green, code 145C.

Spectral characteristics

Spectral analysis showed that in the extracts from inflorescences in all the cultivars and lines, 'Faro' and 'Titicaca', the presence of flavones and flavonols were within the wavelength range from 319 to 334 nm (tab. 3). The original cultivars 'Faro' and 'Titicaca' were revealed the highest absorption maxima, 7 and 4, respectively, as compared to the cultivars selected. The other lines of the 'Faro' group demonstrated 2 to 3 absorption maxima, whereas 'Titicaca White' and 'Titicaca Red' – 2 absorption maxima (tab. 3).

Table 2. Morphological characteristics of *Chenopodium quinoa* Willd. 'Faro' and 'Titicaca' cultivars and their lines

Cultivar/ line	Lenght of shoot (cm)	Number of leaves	Inflorescences		Colour code RHSCC
			lenght (cm)	width (cm)	
'Faro' original	109.2ab*	24.6a	26.4b	5.8ab	Yellow – Green 141 D
'Faro White'	99.8b	23.6ab	28.6ab	5.1b	Yellow – Green 145 C
'Faro Orange'	114.8ab	23.2b	29.4b	5.4b	Orange – Red 34 / 145 B
'Faro Red'	115.2a	20.6b	36.8a	7.4a	Yellow – Green 145 C/ Red –Purple 65 B
'Titicaca' original	72.8d	11.6d	28.3b	4.6b	Yellow – Orange 22 A
'Titicaca White'	82.9c	14.2c	25.1b	4.1b	Yellow 2 C
'Titicaca Red'	77.9d	13.8d	24.7b	4.3b	Yellow – Green 145 C/ Red – Purple 65 A

* Values marked in column with different letters differ significantly at $\alpha = 0.05$

Table 3. Absorption maxima and absorbances of pigment extract in 1% HCl in methanol in *Chenopodium quinoa* Willd. 'Faro' and 'Titicaca' and their lines (± standard error)

Wavelength λ_{max} (nm)	Cultivar/ line						
	'Faro' original	'Faro White'	'Faro Orange'	'Faro Red'	'Titicaca' original	'Titicaca White'	'Titicaca Red'
319	0.8062 ±0.08	–	–	–	–	–	–
321	–	–	–	–	–	0.8232 ±0.16	–
330	0.7721 ±0.09	0.8575 ±0.12	0.7949 ±0.09	0.9906 ±0.32	–	–	1.334 ±0.23
334	–	–	–	–	0.3987 ±0.11	–	–
400	0.6147 ±0.21	–	–	–	–	–	–
418	–	0.1989 ±0.05	0.2238 ±0.02	0.1752 ±0.06	–	–	–
419	1.0154 ±0.44	–	–	–	–	0.1370 ±0.05	–
420	–	–	–	–	0.2447 ±0.10	–	0.1550 ±0.03
458	–	–	–	–	0.2197 ±0.09	–	–
478	–	–	0.2113 ±0.11	–	–	–	–
480	–	–	–	–	0.2067 ±0.23	–	–
530	0.0763 ±0.02	–	–	–	–	–	–
567	0.0714 ±0.02	–	–	–	–	–	–
600	0.0755 ±0.02	–	–	–	–	–	–

Table 4. Absorption maxima and absorbances of pigment extract in 100% acetone in *Chenopodium quinoa* Willd. 'Faro' and 'Titicaca' and their lines (± standard error)

Wavelength λ_{max} (nm)	Cultivar/ line						
	'Faro' original	'Faro White'	'Faro Orange'	'Faro Red'	'Titicaca' original	'Titicaca White'	'Titicaca Red'
322	–	–	–	0.7783 ±0.80	–	–	–
325	–	–	–	–	1.4623 ±0.10	–	–
330	–	–	–	–	–	0.2611 ±0.07	–
331	–	0.1635 ±0.03	–	–	–	–	–
334	0.2521 ±0.05	–	0.2560 ±0.09	0.2570 ±0.07	–	–	–
336	–	–	–	–	–	–	0.2174 ±0.08
375	0.2307 ±0.06	–	–	–	–	–	–
409	0.2462 ±0.19	–	–	–	–	–	–
430	–	–	–	–	0.1669 ±0.01	0.1469 ±0.06	–
431	0.3291 ±0.14	0.0636 ±0.01	0.1375 ±0.06	0.1398 ±0.05	–	–	–
455	0.2040 ±0.83	–	–	–	–	–	–

All the cultivars and lines are characterized also by a maximum absorption in the wavelength range from 400 to 600 nm. The 'Faro' group demonstrates a maximum wavelength λ = 418 nm (except for 'Faro' original λ = 419 nm) and an additional maximum at λ = 400 nm. In contrast, the 'Titicaca' group shows a maximum absorption at λ = 420 nm (except for the 'Titicaca White' λ = 419 nm).

Interestingly, additional absorption at the wavelength λ = 458 nm is found for cultivar 'Titicaca' original, λ = 478 nm for 'Faro Orange', λ = 480 nm for 'Titicaca' original and 3 absorption maxima (λ = 530, λ = 567 nm, λ = 600 nm) for 'Faro' original.

The spectral characteristics of 100% acetone extracts also demonstrated that in all the cultivars and their lines, except for 'Titicaca Red', the presence of carotenoids can be observed, as evidenced by the absorption maxima at wavelength λ = 431 nm for all the cultivars of the 'Faro' group and for λ = 430 nm for the 'Titicaca' original and 'Titicaca White'. Only 'Titicaca Red' cultivar carotenoids were found to indicate a red colour due to the presence of another group of pigments with a maximum of λ = 420 nm (anthocyanins or betacyanins). The presence of flavones and flavonols was also found in the wavelength range from 322 to 336 nm. The 'Faro' group was characterized by the presence of maximum absorption at λ = 334 nm (except for 'Faro White' λ = 331 nm). 'Titicaca' group is more diverse in terms of absorption maxima: each cultivar has a characteristic maximum absorption at different wavelengths (tab. 4).

Molecular markers characteristics

A total number of loci indicated with RAPD for 'Faro' group and 'Titicaca' group were 64 and 75, respectively; with 61% for 'Faro' group as well as 80% for 'Titicaca' group being polymorphic. The highest number of indicated loci was recorded with primer G (13 loci, both for 'Faro' and 'Titicaca' groups), primers B and F gave uniform banding pattern with no polymorphism detected. The average number of loci indicated with RAPD primers was 7.1 and 8.3 per primer in 'Faro' and 'Titicaca' groups, respectively.

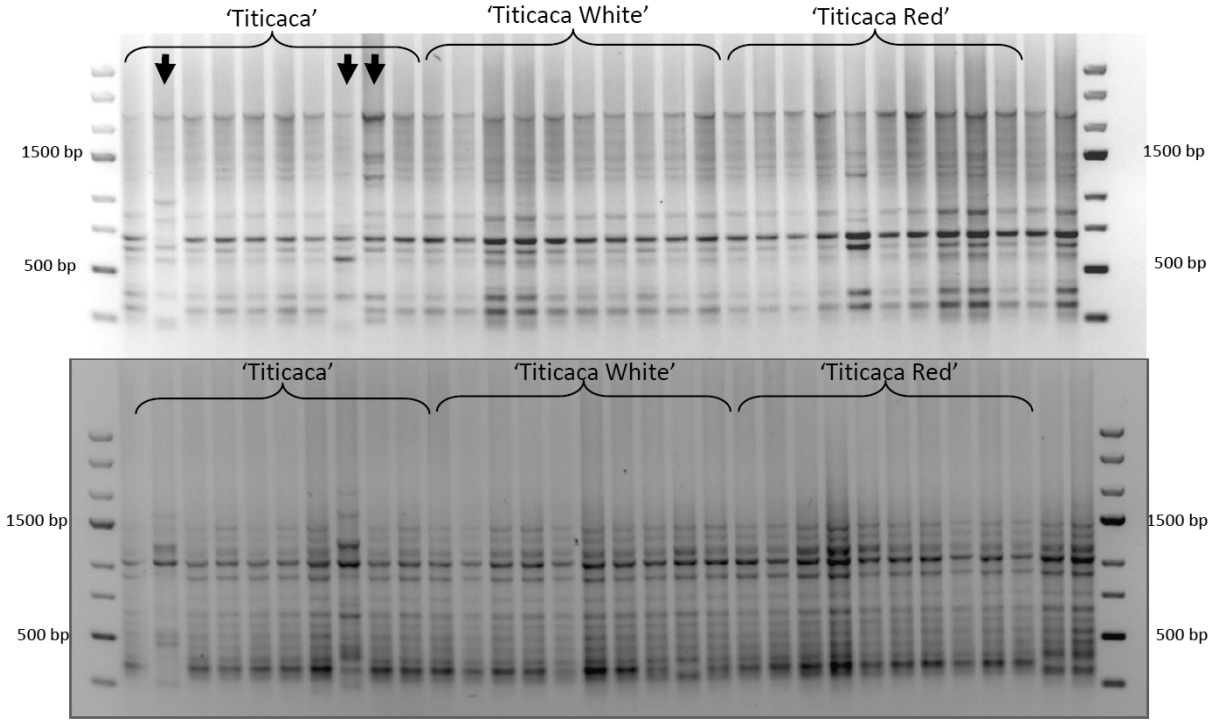

Fig. 2. Banding pattern produced with RAPD primer D (upper) and SCoT 3 (bottom) for 'Titicaca'; outermost lanes are bp ladders; arrows indicate T2, T8 and T9 lanes

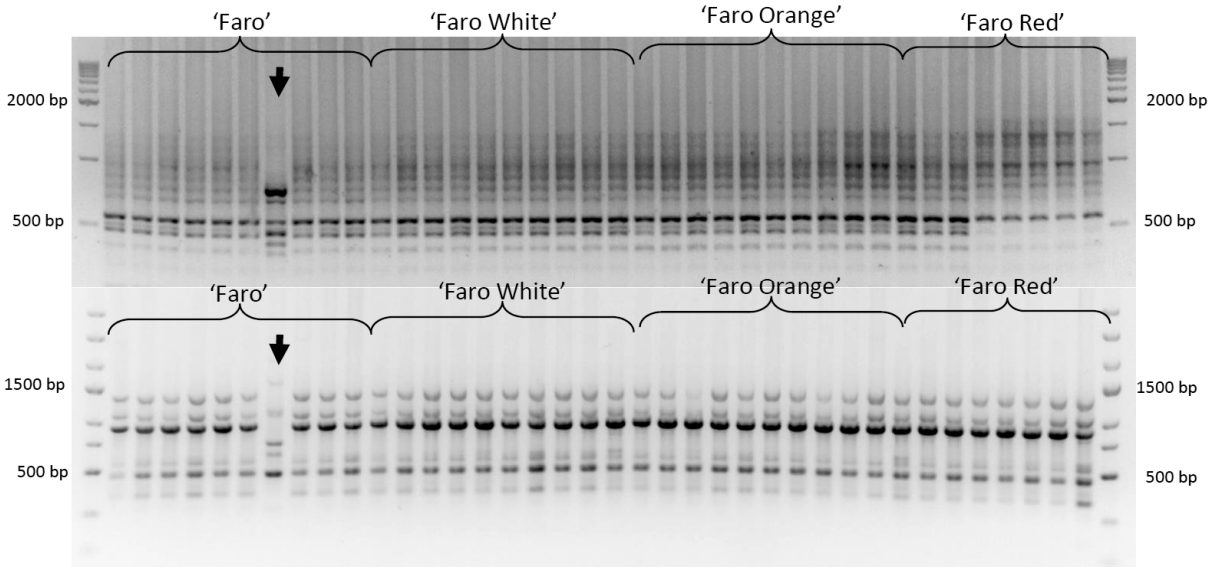

Fig. 3. Banding pattern gained with RAPD primer J (upper) and SCoT 25 (bottom) for 'Faro'; outermost lanes are bp ladders; arrows indicates F7 lanes

Table 5. Genetic distance (%) between variants of 'Titicaca' original cultivar and plants representing predominant banding pattern in 'Titicaca' group as indicated with RAPD and SCoT (in brackets) molecular marker systems

	Predominant	T2	T8
Predominant	0		
T2	51.7 (55.9)	0	
T8	25.3 (43.5)	54.8 (30.5)	0
T9	7.5	53.5	28.9

A total number of loci indicated with SCoT marker system for 'Faro' and 'Titicaca' groups were 79 and 99, respectively, with 75.9% for 'Faro' group and 78.8% for 'Titicaca' group being polymorphic. The highest number of loci was recorded with primer SCoT 3 (17 loci for 'Titicaca' group) and SCoT 33 (12 loci for 'Faro' group). The average number of the loci indicated with SCoT primers was 8.8 and 11.0 per primer in 'Faro' and 'Titicaca' groups, respectively.

For 'Faro' as well as for 'Titicaca' group, the genetic variation detected with molecular markers was indicated only in the original cultivars, while in all of the derivative cultivars no alteration from the predominant banding patterns was detected. It can be concluded that plants representing the selected five derivative lines are genetically uniform within lines (figs 2 and 3).

With the RAPD analysis it was found that three (labelled T2, T8 and T9), out of ten plants representing the 'Titicaca' original cultivar, showed a different banding pattern from the remaining seven plants as well as one from another. With the SCoT molecular markers only the T2 and T8 demonstrated differences as compared to the predominant banding pattern, however, the genetic distances indicated with the SCoT technique were higher (tab. 5).

For the 'Faro' group it was shown both with RAPD and SCoT techniques that only a single plant labelled F7 represented a different banding than all the remaining plants analysed. The genetic distance between F7 and the rest of the plants accounted for 34.1 % and 61.2% in RAPD and SCoT analysis, respectively.

DISCUSSION

Spectral analysis of intact florets of chrysanthemum cultivars, was performed by Kawase and Tsukamoto [1974, 1976]. Lema-Rumińska and Zalewska [2005] investigated the occurrence of extracted flavonoids and carotenoids in 10 mutants of Lady *Chrysanthemum* × *grandiflorum* [Ramat./Kitam.]. The studies revealed that different radiomutants derived from the original cultivar 'Richmond' differed from one another in terms of quality and quantity of flavonoids and carotenoids in inflorescences during flowering. The authors also pointed out a possibility of identifying cultivars based on differences in the occurrence of specific pigments isolated from inflorescences of plants grown under the same environmental conditions.

The first report on betacyanins identification in quinoa seeds appeared in 2015 [Tang et al. 2015]. Sawicki et al. [2016] determined the wavelength range of batacyanins and their derivatives (433–539 nm) and bataxanthins and their derivatives (452–475 nm). In the absorption spectra investigated, we found the presence of absorption maxima in those ranges, which may indicate the presence of betacyanins in the quinoa cultivars tested. Tang et al. [2015] indicate in quinoa the wavelength characteristic for betanin absorption maxima at λ_{max} = 536 nm and isobetanin at λ_{max} = 538 nm. The 'Faro' original had a maximum absorption at wavelength λ_{max} = 530 nm.

However, it is difficult to provide a definite statement which group of pigments it is the maximum for since anthocyanins and betacyanines show an absorption maximum at the same wavelength.

Gonçalves et al. [2012] indicate the wavelength λ_{max} = 478 nm for betaxanthins, as previously stated by von Elbe [2001]. Maximum absorption was achieved in the 'Faro Orange' cultivar.

The morphological characteristics of the *Chenopodium quinoa* (Willd.) cultivars tested showed differences between those cultivars and the original cultivars. These differences concerned both the shoot length, leaf number, inflorescence length and width and the plant colour determined with the RHSCC chart. Most similar in terms of inflorescence colour were 'Faro Red' and 'Titicaca Red' cultivars, different only in the shade of colour code Red-Purple 65A or 65B. The spectral analysis of pigment extracts showed, however, different positions of the absorption maxima demonstrating a distinct variation of these lines. As the only one of the cultivars tested, 'Titicaca Red' had neither a maximum absorption for carotenoids at wavelength λ_{max} = 430 nm characteristic for the 'Titicaca' group nor the maximum at λ_{max} = 431 nm characteristic for the 'Faro' group. However, it recorded a characteristic maximum at λ_{max} = 336 nm for acetone extract. Additional differences in the position of absorption maxima are visible between these cultivars in the absorption spectrum for methanol extracts. The cultivar line 'Faro Red' shows a characteristic maximum at λ_{max} = 418 nm while 'Titicaca Red' at λ_{max} = 420 nm. The differences in the position of absorption maxima may provide a springboard for confirming a cultivar identity and cultivar identification. The new quinoa cultivars and lines were also subjected to tests, showing different physicochemical seed properties [Gozdecka et al. 2015].

The genetic stability of plants representing the lines derived from original 'Faro' and 'Titicaca' has been confirmed with RAPD as well as SCoT molecular markers. The only variation detected with the genetic markers was observed in plants representing original cultivars. RAPD markers were successfully used in the genetic linkage map creation for quinoa [Maughan et al. 2004], although among 60 primers screened only 6 gave polymorphic bands in the plants tested, and the authors pointed to a lower efficiency of this technique than AFLP or SSR markers. The technique has been successfully applied in the genetic diversity and stability studies on chrysanthemum, *Pittosporum ericarpum*, *Cleome gynandra* and more [Rathore et al. 2014, Feng et al. 2016, Thakur et al. 2016]. In our study SCoT technique proved to be more informative since it produced more loci (bands) than RAPD. With both techniques no genetic diversity was recorded for the new lines. Polymorphism detected in plants representing the original cultivars was present in the same plants as revealed by RAPD and SCoT, however the genetic distances between the variant plants and the predominant banding pattern were higher as indicated with SCoT markers for both 'Faro' and 'Titicaca'.

CONCLUSIONS

1. Analysis of morphological features of plants and spectral analysis of pigments extracted from plant inflorescences in *Chenopodium quinoa* Willd. gives an opportunity to confirm the cultivar identity and identification of 'Faro' and 'Titicaca' cultivars and their new lines.

2. Absorption spectra analysis is an effective method of identifying cultivar and it should be applied in practice for an identification of cultivars or cultivars lines.

3. Analysis of molecular markers, indicated by RAPD as well as SCoT technique, revealed a high genetic stability of the new lines of 'Faro' and 'Titicaca', while some variation has been detected across the plants representing original cultivars.

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