BSL 1: Biotechnological tools for improvement of black nightshade (*Solanum nigrum* L. complex), valuable medicinal and vegetable plants in Kenya

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Abstract

Solanum nigrum complex is a group of plant species used as indigenous vegetables but also as traditional medicinal plants in Kenya and other parts of the world. In Kenya, just like in most African countries, both the unripe fruits and leaves are used to cure different ailments. This vegetable is said to improve the CD4 count in HIV patients and all HIV patients are encouraged to take it as part of their diet. Despite that high value, African nightshade is a neglected crop. Up to now the farmers exploit traditional landraces and accessions. For development of improved African *S. nigrum* varieties, knowledge is necessary about the genetic structure of the local African nightshade accessions. Amplified fragment length polymorphism (AFLP) technique was performed to discriminate accessions from the Western region of Kenya. Production of haploid plants of the *S. nigrum* complex and subsequent chromsome doubling is a promising tool to obtain pure inbred lines in a short time. Therefore, in this study, anthers of *S. nigrum* were cultivated in vitro. It was observed that the microspores underwent the first divisions and calluses were formed.

Keywords: AFLP, African nightshade, anther culture, flowcytometry, neglected crops

Introduction

There are a number of species in the *Solanum nigrum* complex which include *S. nigrum, S. villosum, S. scabrum, S. americana, S. burkankii,* and *S. schenopodioides* among others. The *S. nigrum* complex which refers to all of the dozens of black nightshade species around the world formally called *S. nigrum* comprises of both native and bred *Solanum* species used as vegetables and source of fruits in Kenya and other parts of the world. These plants are believed to have a high nutritional value (Schippers, 2000). It has been reported that the nutrient content of this vegetable can provide 100 % of the recommended daily allowance for an adult for calcium, iron, b-carotene, and ascorbic acid and 40 % of protein if 100 g of the fresh vegetable is consumed (Abukutsa-Onyango, 2003). The high nutritional value makes African nightshade especially important for poor people. Demand for the vegetable has increased in the urban areas of Kenya making it a cash crop (Ojiewo et al., 2013).

Medicinally, African nightshades are used for stomach upsets, duodenal ulcers, and swollen glands (Edmonds and Chweya, 1997; K'opondo et al., 2005). They are also squeezed on babies' gums to ease pain during teething. The HIV patients are given this vegetable together with anti-retroviral drugs at the AMPATH in Eldoret, Kenya (http://www.ampathkenya.org/). Studies on in vitro antiviral activity of *S. nigrum* against Hepatitis C Virus by Javed et al. (2011) showed that methanol and chloroform extracts of *S. nigrum* seeds exhibited 37 % and more than 50 % inhibition of Hepatitis C Virus replication, respectively.

In Western Kenya, three species have been identified namely *S. scabrum, S. nigrum* and *S. villosum*. These species have shown significant antimicrobial activities on different microbes of both humans and plants (Matasyoh et al., 2014). The work showed that the *S. villosum* had the best activity against numerous bacterial species, among others *Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli* and *Bacillus subtilis.* These microbes are very important because they cause diseases like typhoid, pneumonia, ring worms, mouth ulcers, diarrhoea etc which affect most poor people who cannot afford nor access modern medication. *S. scabrum* showed best antifungal results against all the *Fusarium* species which affect crops.

The knowledge that the *S. nigrum* complex has high nutritional and medicinal benefits has led to an increase in their consumption, monetary value and demand in Africa. There is a high demand to improve *S. nigrum* cultivars. The production of doubled haploid plants ensures that homozygous lines can be obtained in a shorter period, unlike conventional breeding which takes 5 - 6 years of selfing to achieve this homozygosity level. Through development of haploids and later doubled haploids, cross breeding process can be initiated. Here we present the first results of anther culture of the Kenyan *S. nigrum* complex accessions. Furthermore, for development of new African *S. nigrum* varieties knowledge is necessary about the genetic structure of the local African nightshade accessions. For that purpose amplified fragment length polymorphism (AFLP) technique was used.

Materials and Methods

Plant material

Twelve accessions belonging to the *Solanum nigrum* complex (*S. scabrum, S. nigrum* and *S. villosum*) were used as anther donors and for DNA extraction. Seeds of the *S. nigrum* complex were obtained from the farmers from Kenya, and the Germany seeds were obtained from the garden at the Julius Kühn Institute, Quedlinburg (Table 1, Fig. 1). They were grown in pots in a greenhouse providing standard horticultural conditions from March to August.

Tab. 1 Used accessions of Solanum nigrum complex from Kenya and Quedlinburg, Germany.

Number	Species	Description, origin
1	*S. scabrum*	Indigenous species, Kabras, Kakamega
2	*S. scabrum*	Improved species, Bungoma
3	*S. scabrum*	Improved variety, Botsotso, Kakamega
4	*S. scabrum*	AMPATH seeds grown in Kakamega
5	*S. scabrum*	Species, Eldoret
6	*S. scabrum*	Species, Ahero, Kisumu
7	*S. villosum*	Indigenous species, Eldoret
8	*S. villosum*	Indigenous species, Bungoma
9	*S. nigrum*	Indigenous species, Kabras, Kakamega
10	*S. nigrum*	Indigenous species, Bungoma
11	*S. nigrum*	Quedlinburg, Germany
12	*S. nigrum*	Quedlinburg, Germany

Anther culture, flowcytometry, and AFLP analysis

Flower buds were surface sterilized by submerging them in a NaClO solution (3 % active chlor) for 15 min, prior to three washes with sterile water. The anthers were excised from the flower buds, and cultured on 35 mm Petri-dishes containing 3 ml liquid medium either K0 + S with 4 mg/l 2,4 D, 1 mg/l zeatin (KELLER et al., 1975), medium N6 or EPM + 0.2 mg/l 2,4 D (CHU, 1978, modified), respectively. Anthers were initially treated with 6 °C or 30 °C for three days, or 32 °C for one day and further cultivated at 25 °C in darkness. At least 50 anthers per variant were tested. Anther callus was transferred to solid regeneration medium (MS + 2.0 mg/l kinetin, 0.5 mg/l IAA, 5 % coconut water, 800 mg/l glutamine, 2 % sucrose) and cultivated under light conditions; 16 hrs light / 8 hrs dark at 25 °C.

Fig. 1 Plants of the S. nigrum complex in greenhouse.

For estimation of the DNA content of plants and callus material was cut with a sharp razor blade in Galbraith buffer (GALBRAITH, 1983). The filtered suspension of nuclei was stained with propidium iodide. Measurement was done using BD FACSCalibur™. AFLP analysis was described in MATASYOH et al. (2015).

Results

Anthers from various accessions of *S. nigrum* complex have shown a high responsiveness to all three induction media (Fig. 2a). It was impossible to decide if the callus was developed from somatic anther tissue or from microspospores. Although few anthers showed open thecae with callus (Fig. 2b) and first divisions of microspores were found (Fig. 2c). The highest callus induction (100 %) was observed after 30 °C treatment on medium EPM for accession 10 (*S. nigrum* species indigenious from Bungoma). The response to induction medium was genotype depending. While for *S. scabrum* accessions the highest callus induction frequency was noticed on K0 + S for *S. nigrum* and *S. villosum* EPM medium was the best (Table 2).

Androgenetic calluses were further cultivated on several media with the aim to induce shoots. Calluses are growing over two years but regeneration could not be induced.

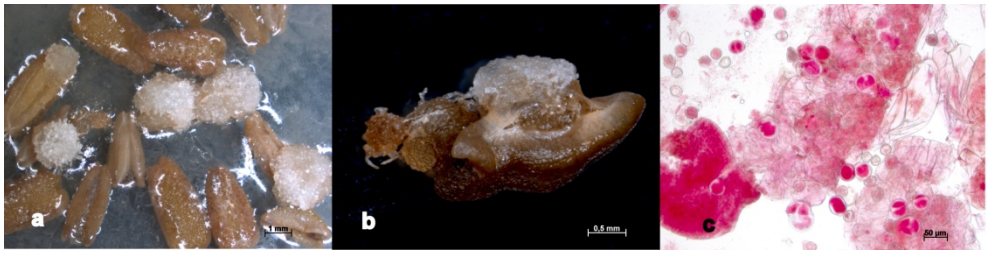

Fig. 2: Development of anthers in vitro, a: Callus on anthers Solanum scabrum on medium K0 + S after four weeks, b: Anther with open theca and callus, c: Divisions of microspores of S. nigrum, one week on EPM.

Tab. 2 Survey about anther culture

Genotype	Induction medium	number of cultivated anthers	Number of calluses	Regeneration frequency (%)
Solanum. scabrum	EPM	200	1	0.5
Accessions 1, 4, 5, 6	K0+S	200	34	17.0
	N6	200	6	3.0
S. nigrum	EPM	500	300	60.0
Accession 10	K0+S	300	28	9.3
	N6	350	85	24.3
S. villosum	EPM	200	62	31.0
Accessions 7, 8	K0+S	300	35	11.7
	N6	250	60	24.0

Generally flowcytometry showed the same DNA content of calluses as the donor plant. One callus from accession 10 revealed the haploid DNA content indicating that a further development from haploid cells is feasible.

By AFLP analyses the twelve *Solanum* accessions were clearly distinguishable (MATASYOH et al., 2015). *S. villosum* was the most divergent accession. *S. nigrum* and *S. scabrum* accessions were near up to intermixing in the cluster analysis. This underpins the difficulty to classify the accessions of the *S. nigrum* complex.

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