

## A NEW VARIETY OF *SOLANUM SURATTENSE* BURM. FROM PAKISTAN

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### Abstract

*Solanum surattense* is phenotypically highly polymorphic species. This polymorphism adds difficulty to identification of lower order taxa of this species. The present study was carried out to find morphological, biochemical and molecular markers for the proper identification of various lower taxa of *Solanum surattense*. Herbarium and fresh samples were subjected to morphological, biochemical and molecular studies. The present investigation suggested a new variety of *Solanum surattense*. The distinguishing morphological markers of *S. surattense* var. *awanicum* are white colour of flower, 4-5 stamens, green colour anthers and stellate hairs on style and ovary. The variety showed marked biochemical and molecular difference from the type species. A new variety of *Solanum surattense* has been proposed as *Solanum surattense* var. *awanicum* Zubaida Yousaf & Mir Ajab Khan.

### Introduction

*Solanum surattense* is a medicinally important prostrate herb with scattered stellate hairs and prickly stem. This plant has high concentration of solasodine, a starting material for the manufacture of cortisone (Heiser, 1969). It grows widely in Pakistan up to 1300m and also in other parts of world under the similar climatic and geographical conditions (Stewart, 1972; Nasir, 1985).

*S. surattense* is the taxon of the subgenus *Leptostemonum* and sections *Melongena* (Schonbeck-Temesy, 1972). It can be easily distinguished from the other taxa of the section *Melongena* by prostrate habit, the presence of prickles extensively on leaves and stem, lobed leaves, scattered stellate hairs and discoid seeds. The nomenclature of this species remained controversial in the past. Burmanii (1768) described it first of all and gave the name of *S. surattense*. Later on taxonomist of different era gave it various names. The most commonly used synonym of *S. surattense* is *S. xanthocarpum*. Schard & Wendelbo gave this name in (1795) followed by Clarke (1885), Rechinger (1958) and Cooke (1967). Now the valid taxonomic name is *S. surattense* Burm (Nasir, 1985).

Phenotypically this species is highly polymorphic. Morphological characters such as point of origin of spines, arrangement of spines, presence of hairs on petiole and stem, flower colour, length of filaments. Length of style and presence of stellate hairs on ovary showed polymorphism. Most commonly found flower colour is purplish but white colour flowers are occasionally present (Nasir, 1985). This phenotypical polymorphism leads to the confusion for the identification of lower order taxa of *S. surattense*. The aim of the present study was to identify morphological and biochemical markers which could help in the identification and to determine taxonomic status of variant of *Solanum surattense*.

### Materials and Methods

**Morphological studies:** Preserved specimens of *Solanum surattense* from the National Herbarium, National Agricultural Research Center (NARC) Islamabad, Quaid-i-Azam

University Herbarium Islamabad (ISL) and Pakistan Museum of Natural History (PMNH) Islamabad were studied. Fresh specimens were also collected and mounted on herbarium sheets for study. The morphological characters of all the available specimens of *Solanum surattense* were studied under the dissecting microscope (10x / 21 D). Each measurement of a specimen was taken 3-5 times to ensure the reading and calculated its mean. Total morphological characters of a specimen, observed were 64. The selection of taxonomic characters was done by following Schonbeck-Temesy (1972) and Nasir (1985).

**Biochemical studies:** Total seed protein was extracted from 0.01g of seed flour using 400  $\mu$ l of extraction buffer that contains 0.05M Tris-HCl pH 8.0, 0.2% SDS, 5M urea, 1% Mercaptoethanol. Seed flour was thoroughly mixed with buffer solution by vortexing. The extracted protein was separated by centrifuging the sample @ 15000rpm for 10 mins. Electrophoresis was carried out in the discontinuous SDS-PAGE system of Laemmli (1970) using 15% acrylamide gel. Electrophoresis was run at 100V. The gels were stained in the staining sol (440ml methanol, 60ml acetic acid, 500ml distilled water and 2.25g of coomassie brilliant blue) for almost 45mins. Destaining was done in a solution containing 200ml methanol, 50ml acetic acid and 750ml of distilled water until the background color disappeared and protein bands were clearly visible.

**Molecular studies (RAPD analysis):** The seeds of 3 different specimens of *Solanum surattense* with morphological differences collected from field and were sown in small plastic pots in green house. Pots were regularly irrigated till the plants reached 3 - 4 leaves growth stage. DNA was extracted from leaves by using modified method of Murray & Thompson (1980). Polymerase Chain Reaction (PCR) was performed using reaction mixture 15 $\mu$ l contained 20mM Tris-HCl (pH 7.8), 100 mM KCl, 3mM MgCl<sub>2</sub>, 200uM of each dNTP, 1uM Primer, 50ng of DNA and one unit of *Taq* DNA polymerase. Ten base pair OPERON primers (OPG-1 to OPG-5 and OPG-10, OPA-1 to OPA-15 and OPA-18) and Gene Link primers (AC-11 to AC-20) were used for RAPD. Amplification reaction was carried out at the Perkin Elmer Thermocycler 480. After PCR, the amplified fragments were separated on 1% agarose gel in 0.5 X TBE buffer, stained by Ethidium bromide, visualized and photographed by using the gel documentation system FAS500 Epi- Light UV. The molecular size of the amplification products was measured with GeneRuler<sup>TM</sup> 100bp DNA ladder. The accessions were scored for the presence or absence of RAPD bands. The presence and absence of the bands was scored in a binary data matrix. Polymorphic bands were scored and used for further analysis.

## Results

*Solanum surattense* Burm. F., *Fl. Ind.*, 57. 1768.

**Synonyms:** *S. armatum* Forssk., *S. virginianum* Jacq., *S. xanthocarpum* Schrad. & Wendl. *S. Jacquinii* Will., *S. diffusum* Roxb.

**Common name:** Spiny nightshade, Yellow fruited nightshade

**Vernacular name:** Kundari, Momoli

**Morphological description of the specimens with purple colour of flower:** Prostrate herb, 19-40cm long, stellate hairs are present, prickly. Stem cylindrical, 0.7-1.4cm in width, greenish brown, much branched. Leaves petiolate, 1.1-2.5cm long, villous and prickly. Lamina 1.4-7cm long, 0.4-4.4cm width, margin lobbed, apex obtuse, base oblique-rounded, mid-rib is not very much prominent. Inflorescence axillary cymose, peduncle 1-2cm long. Flowers purple, 0.8-2cm long, 0.3-0.7cm width, villous. Sepals 5, 0.3-0.5cm long, 0.2-0.3cm width, green, margin hairy, apex acute, pubescent. Petals 5, 0.4-0.7cm long, 0.2-0.4 cm wide, margin hairy undulate, apex obtuse, pubescent. Stamens 5, filament up to 0.1cm long, smaller than anthers, Anthers 0.4-0.6 (0.7) cm long. Style 0.7-1.1cm long, partially hairy, stigma capitate, ovary blackish, rounded. Fruit typically berry, 0.7-1cm long, globose, Yellow with white or green streaks. Seeds discoid, minutely reticulate, whitish yellow in colour, numerous, shiny. (Fig. 1)

**Morphological description of the specimens with white colour of flower:** Prostrate herb, 19-40cm long, stellate hairs are present, prickly. Stem cylindrical, 0.7-1.4cm in width, greenish brown, much branched. Leaves petiolate, 1.1-2.5cm long, villous and prickly. Lamina 1.4-7cm long, 0.4-4.4cm width, margin lobbed, apex obtuse, base oblique-rounded, mid-rib is not very much prominent. Inflorescence axillary cymose, peduncle 1-2cm long. Flowers white, 0.8-2cm long, 0.3-0.7cm width, villous. Sepals 5, 0.3-0.5cm long, 0.2-0.3cm width, green, margin hairy, apex acute, pubescent. Petals 5, 0.4-0.7cm long, 0.2-0.4cm wide, margin hairy undulate, apex obtuse, pubescent. Stamens 4-5, filament up to 0.1cm long, smaller than anthers, Anthers 0.4-0.6 (0.7) cm long. Stamens are longer than carpels, Style 0.7-1.1cm long, partially hairy, stigma capitate, ovary blackish, rounded, stellate hairs are present. Fruit typically berry, 0.7-1cm long, globose, Yellow with white or green streaks. Seeds discoid, minutely reticulate, whitish yellow in colour, numerous, shiny (Fig. 2).

**Type specimen:** In insula Zuratta, D. Garcin (G).

**Distribution:** Pakistan (Punjab, NWFP, Azad Kashmir, Baluchistan, Sindh), N. Africa, S.E. Asia, Australia and Polynesia.

**Var. *awanicum*** Zubaida Yousaf, Mir Ajab Khan, Zabta Khan Shinwari spines alternate. Flower white. Stamens 4-5, anther green in colour. Stellate hairs on Style and ovary.

**Holotype:** Rawalpindi district: Tarnol, 8 March 1978, *Abdul Saboor and Manzoor*, 79684 (ISL).

**Biochemical studies:** Total number of protein bands observed in protein profile of different specimens of *S. surattense* is 14. The protein profile of *S. surattense* (W) consists of 10 bands whereas 12 bands comprise the profile of *S. surattense* (P1 &P2). The *S. surattense* (P1 and P2) are almost similar in their protein profile. The intensity and number of bands are same in both specimens (Fig. 3). Whereas *S. surattense* (P1 & P2) and *S. surattense* (W) differ from each other based on intensity and number of protein bands. Band 5 and 7 is present in the profile of *S. surattense* (W) but absent from the profile of other two samples. Similarly band 1, 6, 8 and 9 are the part of protein profile of *S. surattense* (P1 &P2) and absent from *S. surattense* (W). The difference also lies in the intensities of the bands. Band 4 is of low intensity in *S. surattense* (W) but of intermediate in *S. surattense* (P1 & P2).



Fig. 1. *Solanum surattense* Burnm with purple flowers.

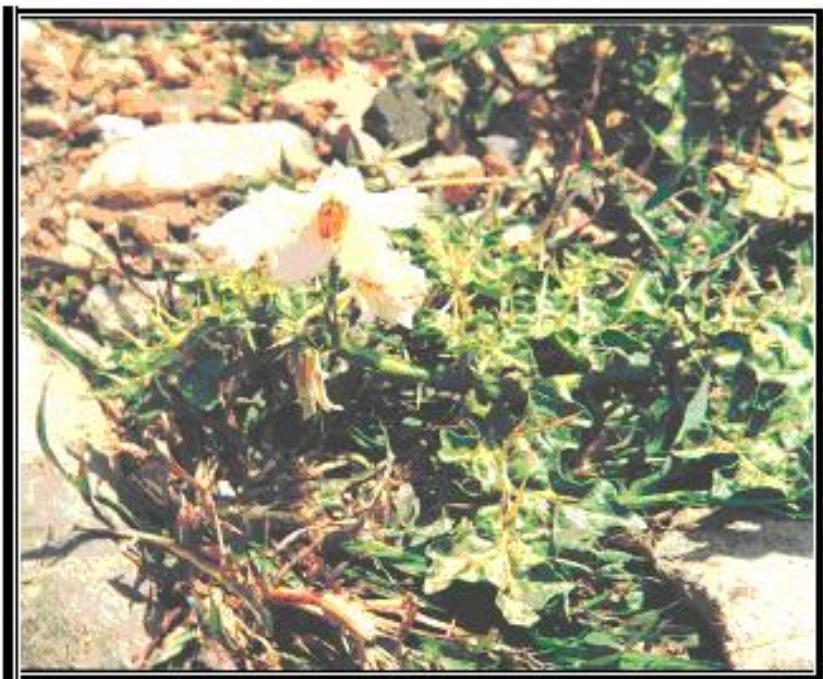


Fig. 2. *Solanum surattense* Burnm with purple flowers.

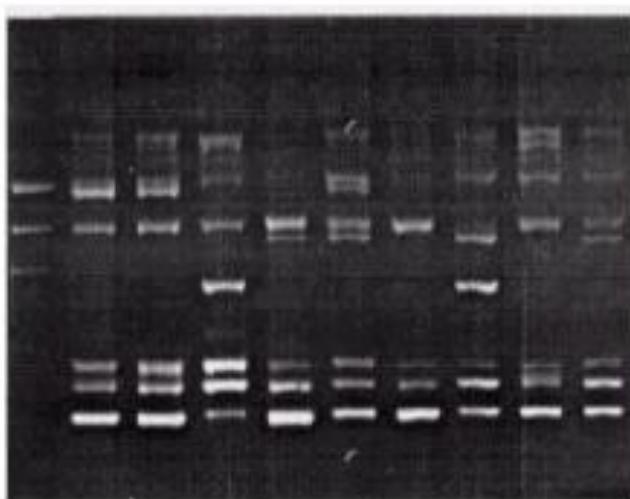
<i>Bands NO.</i>	<i>Mol.wts (Kda)</i>	1	3	4
1	33		■■■■■	■■■■■
2	32	■■■■■	■■■■■	■■■■■
3	30	■■■■■	■■■■■	■■■■■
4	29	■■■■■	■■■■■	■■■■■
5	28	■■■■■		
6	26		■■■■■	■■■■■
7	25	■■■■■		
8	24		■■■■■	■■■■■
9	21		■■■■■	■■■■■
10	20	■■■■■	■■■■■	■■■■■
11	19	■■■■■	■■■■■	■■■■■
12	15	■■■■■	■■■■■	■■■■■
13	12	■■■■■	■■■■■	■■■■■
14	11	■■■■■	■■■■■	■■■■■

Fig. 3. Electropherogram of *Solanum surattense* on 12.25% acrylamide gel.Key: 1= *S. surattense* (W), 2= *S. surattense* (P1), 3= *S. surattense* (P2)

W= White colour flowers, P= Purple colour flowers

■■■■■ Light band ■■■■■ Dark band ■■■■■ Very dark band

1 2 3 4 5 6 7 8 9 10

Fig. 4. DNA amplification of *S. surattense* and *S. surattense* var. *awanicum* using primer AC-11Key: 7= *S. surattense* var. *awanicum*, 8= *S. surattense*

**Molecular studies:** Total 30 primers of 10 base oligonucleotide were surveyed to generate polymorphic scorable bands. Of these only 16 primers (AC-11, AC-12, AC-14, AC-15, AC-20, OPG-01, OPG-02, OPG-03, OPG-04, OPG-05, OPG-10, OPA-01, OPA-02, OPA-03, OPA-04 and OPA-05) generated 12 scorable bands, hence used for *S. surattense* DNA finger printing. The amplified DNA product appeared in the range of 1.7 to 0.36 Kb (Fig. 4). The bands beyond this range were not produced clearly. The amplification of DNA with primer AC-11 is shown in Fig 4. Within the species upto 42% of the scored RAPD markers were polymorphic. However the level of polymorphism varied with different primers. The lowest numbers of fragments (2) were observed by primer OPA-02, 04 & 05, whereas highest number (5) by primer AC-11.

## Discussion

The present investigation included morphological, biochemical and molecular studies of *S. surattense*. Intra specifically these 3 markers showed considerable variations. The morphological characters which mostly exhibit the variation were arrangement / point of origin of spine. Flower colour, number of stamens, colour of anthers and presence of hairs on style and ovary. In the specimen accession 79684 flower color is white while the type specimen has purplish blue colour flower. Spines are alternate and one spine arises from one point while in *S. surattense* (P) spines are opposite and more than one spine arises from one point. Stamens are four in number (this is not the permanent characters sometimes five stamens are also present) while in *S. surattense* (P) stamens are five and carpel is usually longer than stamens but in this variety carpel is smaller than stamens. Anthers are of green colour, whereas in the usual pattern this colour is yellow. The end of style and the top of ovary is covered with stellate hairs, however in *S. surattense* (P) style and ovary are smooth.

Three specimen of *S. surattense* with different morphological characters as mentioned above were selected for electrophoresis. The protein profile of *S. surattense* (P1) and *S. surattense* (P2) (both of these two species have purple colour flowers) was almost similar however *S. surattense* (W) (with white colour flowers) showed variation (Fig 3). The similarity indices based on protein marker of *S. surattense* (P1) and *S. surattense* (P2) was 100% whereas *S. surattense* (W) is 82% similar to other two specimens. Therefore specimens labeled as *S. surattense* (P1) and *S. surattense* (P2) could not be separated. It becomes obvious that point of origin of spines, arrangement of spines, length of filament and style are not enough markers for the differentiation of taxas. The result of SDS-PAGE favors the importance of flower color, the presence of hairs on stem and petiole, style and ovary for the differentiation of lower order taxa in case of *S. surattense*. The genetic variation was also observed in these specimens through RAPD. All the three markers (morphological, biochemical and molecular) strongly favors that *S. surattense* (W) should be given a separate rank. As the both specimens were collected from the same geographical area and have high genetic and molecular similarities therefore it should be treated as the variety of the *S. surattense*.

## References

Burmanii, N.L. 1768. *Flora Indica. Cornelius Haek, Leiden, Johannes Schreud* Amsterdam. pp. 57.  
 Clarke, C.B. 1885. Solanaceae. In: *Flora of British India*, Vol. IV. (Eds.): D. Hookers. Bishen Singh and Mahendra Pal Singh (Publishers), New connaught Place, Dehra, Dun. pp. 236.  
 Cooke, R.J. 1967. *Flora of Presd. Bomb.* 2nd edition., Bombay press, India. pp. 348.

Heiser, C.B. 1969. The Wonder berry. In: *Nightshades-Paradoxical Plants*. (Ed.): W.H. Freeman. Freeman and Co, San Francisco. pp. 62-105.

Laemmli, U. K. 1970. Cleavage of structural protein during the assembly of the head of *Bacteriophage T4*. *Nature*, 680-685.

Murray, G.M. and W.F. Thompson. 1980. Rapid isolation of high molecular weight plant DNA. *Nucl. Acid. Res.*, 8: 4321-4325.

Nasir, J.Y. 1985. Solanaceae. In: *Flora of Pakistan*. (Eds.): S.I. Ali and E. Nasir. Fascicle 168: 1-61. Pakistan Agricultural Research council, Islamabad.

Rechinger, K.H. 1958. Solanaceae. In: *Symbolae Afghanicae*. (Eds.): Koie and K. H. Rechinger. Kommission hos Ejnar Munksgaard (Publishers). pp. 85-88.

Schenobeck-Temesy, E. 1972. Solanaceae. In: *Flora Iranica*. (Ed.): K.H. Rechinger. pp. 1-82. Akademische Druck-u. Verlagsanstalt, Graz, Austria.

Stewart, R.R. 1972. An Annotated Catalogue of Vascular Plants from West Pakistan and Kashmir. In: *Flora of West Pakistan*. (Eds.): E. Nasir and S.I. Ali. pp. 638-645. Karachi University, Karachi.

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