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The germination biology and the pattern of seedling phenology of medicinally important plant *Solanum Surattense* Burm. F.

Sutapa Pal and Pankaj K. Pal*

Department of Botany, Centre for Advanced Studies (UGC),
The University of Burdwan, Burdwan - 713104, India.
*Corresponding author's e-mail: pkpalbot@gmail.com

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ABSTRACT

The seeds of *Solanum surattense* are characterized by a sort of coat imposed primary dormancy which can successfully be overcome by the treatment with 30 N H_2SO_4 for 2 minutes and 250 µg/ml GA3 for 12 hours duration which enhancing the germination to more or less 99%. Seedling of the species typically comprises a pair of flat, relatively thick, green, photosynthetic cotyledonary leaves (paracolyledons), two cataphylls which differ architecturally from the typical foliage leaves (eophylls) and seven to eight foliage leaves. At ±12 days after radical emergence, commencement of the seedling phase takes place by the emergence of plumule as a minute projection (<1mm) at the junction of the two paracotyledons. The first internode differentiates at 3-5 'days after plumule emergence' (DAPE), when the first eophyll is noticed to emerge as a small linear structure. At 4-6 DAPE, the first internode becomes a little less than 1 mm in length subtending the ovate, slightly reflexed first foliage leaf differentiated into a distinct petiole and lamina with a prominent midrib. At 47-49 DAPE, the cotyledonary leaf and the first eophyll get detached from the plant. Shedding of the second eophyll at 59-61 DAPE marks the completion of the seedling phase and onset of the adulthood when the plantlet appears as a miniature of an adult individual.

Key words: Eophylls, Paracotyledons, Seed germination, Seedling phenology, *Solanum surattense*.

INTRODUCTION

The seedling is a very juvenile stage of a plant produced from seed through the process of germination. Apart from its size, the seedling of a plant differs from the adult one in several aspects. A dicotyledonous seedling typically comprises of root, hypocotyl, a pair of cotyledonary leaves and a plumular bud with subsequent internodes and eophylls. The completion of seed germination, marked by the emergence of plumule, may be regarded as the commencement of the seedling phase of a plant. The seedling phase lasts until the young plant assumes the form of the miniature of an adult individual.

Phenological patterns of seedlings have been ascribed to the genetic factors and are often species-specific (Holdsworth et al. 2001, Ackerly 2004, Wang et al. 2009). Developmental processes of seedlings are critical for their establishment and thereby for the survival of the species in a constantly changing environment (Oyelana 2011). Phenological patterns of seedling are therefore of high significance in understanding the reproductive ecology of sexually reproducing species.

On germination, the majority of dicotyledons confirm to either of the two patterns of development. The cotyledons either emerge from the testa to serve as photosynthetic organs or they remain enclosed. While the cotyledons emerge from the testa, the

seedlings are described as phanerocotylar and when they have not, as cryptocotylar (Duke 1965). In some species, the cotyledonary leaves in seedlings become flat, green coloured photosynthetic structures approaching the foliage leaves. Such a cotyledonary leaf is referred to as a paracotyledon (Duke 1965). Seedlings of a vast majority of species, above their cotyledonary leaves, give rise to one or more cataphylls which differ architecturally from the typical foliage leaves. These type of leaves are referred to as eophylls (Duke 1965).

Solanum surattense Burm. f. (= *Solanum xanthocarpum* Schrad and Wendl.) known as "Kantakari" in Bengali vernacular, belonging to the Family Solanaceae, is an important medicinal plant. *S. surattense* is a spiny woody perennial herb of repeatedly branched trailing habit upto 1.2 m tall somewhat woody at its base. Stem is somewhat zigzag, branches numerous, the younger ones tomentose with dense stellate hairs; relatively older branches with straight, shiny prickles. 'Kantakari' has been used traditionally as folk medicine for various purposes (Kirtikar & Basu 1999, Joshi 2000). The plant is thermogenic, anti-helmintic, anti-inflammatory, anodyne, digestive, carminative, appetizer, stomachic, depurative, sudorific, febrifuge, expectorant, laxative, stimulant, rejuvenator, diuretic, emmanagogue and aphrodisiac. Sun dried root of the plant constitutes one of the ten ingredients of the famous ayurvedic medicine, the 'Dasamula'. A paste of the fruits mixed with common salts and the juice of bark of *Shorea robusta* is often used to treat whooping cough in children. The alkaloids, solasonine and solasodine and the steroid, diosgenin have been detected in various parts of the plant (Tupkari 1972, Heble et al. 1967).

MATERIAL AND METHODS

Germination experiments were carried out as per the rules of International Seed Testing Association (1996). The well-known scarification methods viz., hot water treatment and acid scarification with concentrated H_2SO_4, were employed (Copeland & McDonald 2001). Each seed lot containing 100 seeds was allowed to germinate in distilled water on a petri dish (10 cm in diameter). Prior to germination, seeds were rinsed with 0.1% $HgCl_2$ solution for 90 seconds for surface sterilization and then washed thoroughly with double distilled water to the remove traces of $HgCl_2$. The surface sterilized seeds were kept in double distilled water overnight for imbibitions. The petri dishes were placed under diffused natural light at room temperature (27± 2°C) and germination was recorded during the following consecutive days. Each experiment was repeated four times during the entire course of the study. For alternate temperature treatment dry seeds were subjected to hot water (50°C, 55°C, 60°C, 65°C, 70°C, 75°C) for 10 minutes, immediately followed by an exposure to cold water (5°C or 15°C) for another 10 minutes. For scarification, different concentrations of sulphuric acid (18 N, 24 N, 30 N and 36 N) were prepared by using concentrated H_2SO_4 (36 N) and double distilled water. Optimum scarification strategy was deduced by applying those concentrations of H_2SO_4 solutions for variable time durations (1, 2, 4, 8, 10 and 15 minutes). After the scarification treatment, seeds were made acid-free by repeated washing in distilled water. Finally, the seeds were soaked in distilled water followed by sowing in petri dishes for germination. For enhancing the germination (invigoration) properly scarified seeds were treated with GA3 solutions. The GA3 solutions of different dilutions (50, 100, 200 and 250 µg/ml) were applied to the seeds for different durations (12, 24, 48, 72 and 96 hours).

Phenological changes in the seedlings of *S. surattense*, at their different stages of development were primarily observed on those growing in wild habitat. Further details of the developmental changes that take place with respect to the day after plumule emergence (DAPE) were recorded from the naturally grown seedlings in the research plot of Department of Botany, University of Burdwan. Observations were based on a total of 134 seedlings in three consecutive years. Individual seedlings were numbered with small flags and details of the morphological changes that occurred in each of them were noted on a daily basis.

OBSERVATIONS AND DISCUSSION

The freshly harvested seeds of *Solanum surattense* exhibit no germination at all. Tetrazolium

test reveals the existence of viable embryos in more than 99% of the seeds, indicating the presence of a sort of dormancy in them. Scarification with H_2SO_4 imparted positive results, indicating the involvement of the hard seed coat in dormancy. Maximum germination (52%) was obtained in treatment with 30 N H_2SO_4 for 2 minutes, indicating the presence of a sort of innate dormancy in addition to that imparted by the seed coat (Table 1). Germination was enhanced further by treating the properly acid scarified seeds with GA3. Treatment with 250 ìg/ml GA3 for 12 hours duration was most effective for enhancing the germination to more or less 99% (Table 2). Breaking of the innate dormancy to certain extent (52%) by acid scarification and rest by GA3 treatment shows the involvement of seed coat as well as inhibitory chemicals in imparting the dormancy in *S. surattense* seeds. This phenomenon is often referred to as 'Double dormancy' (Kozlowski 1972).

Table 1. Germination percentage of seeds of *Solanum surattense* scarified with different concentrations of sulphuric acid for different time durations.

Treatment with H_2SO_4	Time (minutes)				
	1	2	4	8	15
18 N	2%	7%	28%	10%	12%
24 N	5%	16%	32%	8%	0
30 N	28%	52%	24%	0	0
36 N	21%	16%	10%	0	0

Table 2. Germination percentage of seeds of *Solanum surattense* treated with different concentrations of GA3 for different time durations.

Treatment with GA3 (µg/ml)	Time (hours)				
	12	24	48	72	96
50	48.65%	83.67%	88%	96.67%	92.07%
100	62.67%	86.08%	91.01%	90%	83.33%
200	70%	90%	92.33%	83.08%	80.01%
250	99.88%	96.67%	80%	73.33%	63.33%

A fully imbibed, scarified seed of *S. surattense* in water takes 6-8 days for inception of germination, when the radicle just emerged from the seed coat (Plate 1A).

Seed germination of the species is of epigeal type. After an interval of 3-4 days since inception when the radicle attains a length of ±1 cm, the hypocotyl appers as a hook. The cotyledons together with the plumular bud, all remaining enclosed within the seed coat, are pulled above the ground (Plate 1B).

Subsequently, at 4-5 days after radicle emergence [DARE], the hypocotyl straightens and the cotyledons, still remaining enclosed within the seed coat, is pushed into the air nearly 4 mm above the ground (Plate 1C).

At 6-7 DARE, when the hypocotyl attains a height of ±4mm, proximal parts of the two cotyledonary leaves become exposed due to their growth together with uncoiling. The seed coat is pushed distally. Initially, the cotyledonary leaves are yellowish green in colour and remain fully appressed with each other. Simultaneous with the elongation of the radicle, uncoiling and growth of the pair of cotyledonary leaves continue and their colour gradually turns pale green.

Elongation of hypocotyls continues and at 8-9 DARE, a small slit appears between the bases of opposing cotyledonary leaves, which mark the commencement of their divergence from each other. The slit gradually extends towards apex. At this stage, due to the inrolled lateral margins, the cotyledonary leaves appear somewhat keeled. Distal portions of the two inrolled cotyledonary leaves still remain in contact with each other with the seed coat terminally attached like a cap (Plate 1D-E).

At 10-11 DARE, the keeled, pale green cotyledonary leaves, measuring ± 6 mm x 1mm, become totally separated from each other while the seed coat still remains attached to the tip of either of the two or is shed (Plate 1F).

Subsequently, by ±12 DARE, each of the cotyledonary leaves, in its form, approaches a relatively thick small foliage leaf, being a straight, flattened, structure with a petiole of nearly 1mm length and the linear-lanceolate lamina ± 7 x 2 mm in size, entire margin and a faintly visible midrib. Therefore, the cotyledonary leaves of *S. surattense* are to be regarded as paracotyledons. At this stage, the hypocotyl attains a height of nearly 1cm and the plumule is discernible (<1mm) as a minute projection at the junction of the petioles of two oppositely disposed paracotyledons. It marks the completion of the germination phase and the onset of the seedling phase.

PLATE 1

A. Initiation of the germination of *S. surattense* seed. **B**. A germinating seed, the radicle attaining a length of ±1 cm, the hypocotyl appearing as a hook and the cotyledons together with the plumular bud, remaining enclosed within the seed coat, have been pulled above the ground. **C**. Straightening of the hypocotyl of the germinating seed and the cotyledons, still remaining enclosed within the seed coat have been pushed up nearly 4mm above the ground. **D**. Exposure of the proximal parts of cotyledonary leaves pushing behind the seed coat distally. **E**. Commencement of divergence between the inrolled cotyledonary leaves, marked by the appearance of a small slit between their bases, while their distal portions still touching with each other bearing the seed coat terminally like a cap. **F**. Appearance of the pair of oppositely disposed paracotyledons as thick small foliage leaves, each with a straight, flattened, linear-lanceolate lamina with a faintly visible midrib. **G**. Seedling showing the emergence of first foliage leaf (eophyll-I) in the form of a minute linear structure at the junction of the two paracotyledons. **H**. Seedling at the stage of the emergence of the second foliage leaf (eophyll-II). **I**. Seedling exhibiting the emergence of the third foliage leaf, while the stem becoming reddish in colour. **J**. Seedling showing emergence of the fourth foliage leaf; the laminae of the eophyll -I and eophyll-II reaching to their maximum sizes and becoming ovate in outline with entire to slightly undulated margins. **K**. Seedling showing the emergence of the fifth foliage leaf and the development of various degrees of undulations at the margins of third and fourth foliage leaves. **L**. Seedling showing the commencement of senescence of the cotyledonary leaves as marked by the graying of their terminal portions; appearance of spines on the midribs of the third, fourth and fifth foliage leaves. **M**. Seeddling at the stage exhibiting the appearance of the sixth foliage leaf; shedding of one the cotyledonary leaves, commencement of yellowing of the eophylls. **N**. Seedling showing the final stage by the emergence of the eighth foliage leaf and abscissions of the remaining cotyledonary leaf and the first eophyll. **O**. A young individual soon after its assumption of the adulthood. **P-Q**. flower buds are seen to appear close to the axil of the seventh foliage leaf.

By the next day i.e., at 1 DAPE (Day after Plumule Emergence), emergence of the first foliage leaf is noticed, appearing as a minute linear structure nearly a millimeter in length (Plate 1G). The paracotyledons grow further. The linear-lanceolate lamina of each of the paracotyledons reaches a size of ± 10 x 2.5 mm, exhibiting a rather distinct midrib and faintly visible unforked laterals.

Subsequently, when the second foliage leaf emerges at 4-6 DAPE, the first internode becomes a little less than 1mm in length subtending the ovate, slightly reflexed first foliage leaf differentiated into a distinct petiole and a ± 3 x 2 mm lamina with a prominent midrib. At this stage, the paracotyledons achieve their maximum size, each with a lamina of 11-12 x 3mm and a petiole of nearly 2 mm length (Plate 1H).

Emergence of the third foliage leaf takes place at 14-16 DAPE. At this stage the stem acquires a reddish green colour. The lamina of the first foliage leaf attains a size of 7 x 5 mm with a prominent petiole 4 mm in length. The lamina of the second foliage leaf becomes 6 x 3 mm with a petiole 2 mm in length. At this stage, the nodes are clearly discernible due to the elongation of petioles of subtending leaves in alternate phyllotaxy (Plate 1I).

The fourth foliage leaf emerges at 21-24 DAPE when the shoot reaches a height of 1.2-1.6 cm. The laminae of the first, second and third foliage leaves grow up to a sizes of 11 x 9 mm, 19 x 6 mm and 5 x 3 mm respectively. All the leaves are ovate with entire lateral margins. The first and second foliage leaves exhibit no further growth. Their ovate laminae exhibit entire to slightly undulated margins, subacute to obtuse apices, distinct midribs and faintly visible reticulations. Those first two foliage leaves, in form as well as venation, differ strikingly from the typical foliage leaves of the species as seen on adult individuals. Therefore, those are to be regarded as 'eophylls' (Plate 1J).

Emergence of the fifth foliage leaf takes place at 27-29 DAPE. This is a remarkable stage in view of the development of various degrees of undulations at the margins of third and fourth foliage leaves. The third foliage leaf comprises a 4 mm long petiole and a 16 x 8 mm ovate lamina with obtuse apex, undulated to shallowly lobed margins and quite apparent reticulate venation. The lamina of the fourth foliage leaf is also ovate in shape and 11 x 7mm in size with lobed margins, subacute to obtuse apex and prominent reticulate venation (Plate 1K).

Senescence of the cotyledonary leaves, marked by the greying of their terminal portions, commences at 33-34 DAPE. The third foliage leaf reaches its maximum size with a 6 mm long petiole bearing a 23 x 12 mm, ovate lamina. The lamina of the fourth foliage leaf becomes 21 x 17 mm, terminating a 4 mm long petiole. Appearance of spines on the midribs of the third, fourth and fifth foliage leaves is also a noticeable feature of this stage (Plate 1L).

By the emergence of sixth foliage leaf at 41- 44 DAPE, one of the cotyledonary leaf sheds and commencement of yellowing of the eophylls takes place. The lamina of the fourth foliage leaf reaches its maximum size of 30 x 18 mm and that of the fifth foliage leaf attains a size of 27 x 17 mm (Plate 1M).

At the time of emergence of the seventh foliage leaf at 43-45 DAPE, the lamina of the fifth leaf attains its maximum size of 34 x 22 mm and that of the sixth foliage leaf reaches a size of 10 x 5 mm. Strikingly, the fifth leaf approaches the form of the typical foliage leaves of adult individuals, being characterized by the oblong outline of its lamina, deeply lobed margins, reticulate venation and spines on the midrib as well as on the lateral veins. The features of third and fourth foliage leaves on the other hand are somewhat intermediate between the eophylls and the typical foliage leaves. Therefore, the third and fourth foliage leaves are transitory ones, neither the typical eophylls nor foliage leaves (Plate 1N).

At 47-49 DAPE, emergence of the eighth foliage leaf takes place. By this time, the remaining cotyledonary leaf and the first eophyll gets detached from the plant. At this stage, the lamina of the sixth leaf becomes 35 x 20 mm and that of the seventh leaf becomes 7 x 3 mm (Plate 1O).

Shedding of the second eophyll at 59-61 DAPE marks the completion of the seedling phase and onset of the adulthood when the plantlet appears as a miniature of an adult individual (Plate 1O).

The plant finally attains its reproductive maturity at 74-77 DAPE when the flower buds are seen to appear close to the axil of the seventh foliage leaf (Plate IP-Q).

From the above seedling phenology it may be concluded that the seedling of *S. surattense* consists of root, a hypocotyl, two cotyledons (paracotyledons) and a plumular bud with subsequent internodes and eophylls (juvenile foliage leaf not looking like a mature leaf) and also the mature leaves. The first two foliage leaves are ovate in shape without any prominent reticulation as well as spines therefore they are regarded as eophyll. The third foliage leaf is with wavy margin and with reticulation, but lobation is not so pronounced like in a mature leaf. The fourth leaf has all the characters of the leaf structure like a mature leaf. Hence, the third foliage leaf is regarded as the transitory between eophyll and true leaves.

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