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The Germination Biology and Pattern of Growth in Eight Solanum species Found Endemic in Nigeria

Olatunji Afolabi Oyelana

Department of Biological Sciences, College of Natural Sciences, Redeemer's University, Km 46, Lagos-Ibadan Expressway, Mowe, Ogun State, Nigeria

ABSTRACT

The existence of diverse morphotypes and the constantly expanding genome have made evolutionary assessment of members of the Solanum group difficult. The different patterns of seedling growth following germination provide a set of diagnostic tools for analysing the degree of relatedness among these species. Subsequently, species rate and percentage germination and the developmental patterns of seedlings to 10th leaf stage were assessed. In the control, the germination rate and percentages were 0.94 (85%) and 0.09 (80%) for Solanum gilo and S. melongena Golden, respectively. The latter species had a significant increase in rate of germination from 0.09 to 0.11 and 0.89, respectively in the control, 1% KMnO₄ dilution and mechanical scarification while no significant increase was observed in all the concentrations of colchicine. The radicles emerged 4 days after soaking and cotyledons were fully spread 3-5 days after and subsequently dropped from 8 days of growth. The first three leaves were elliptic and pinnately veined, initiated in bud from 14 days of radicle growth and were shed after 12 days of growth. The 4th and subsequent leaves were initiated in buds from 18-20 days of radicle growth, revealed the net venation and lobbing of margins characteristic of members of the genus and their growth was indeterminate. The lateral buds were initiated in all seedlings and trichome hairs became prominent on the stems of S. scabrum subsp. scabrum from 54 days after radicle growth. The pattern of germination and seedling growth was remarkably similar for all the species. The seed shape, size and colour distinguished the eight species into individuals with ovoid or flattenedreniform seeds which may either be brown, yellow or pale yellow in colour. Flowers were bisexual with exerted styles while few were staminate or short styled with anthers loosely arranged around the styles. The species common ancestry and close evolutionary history were discussed.

Key words: Solanum, germination biology, evolution, colchicine, trichome, phenology

INTRODUCTION

The seasonal life cycle and developmental processes are critical to plant survival and continue existence (Rathcke and Lacey, 1985) in an unstable but constantly changing environment. Endogenous growth regulators (Joseph *et al.*, 2010) including Salicytic Acid (SA) helps to regulate a number of physiological processes in plants from germination to senescence to ensure adaptation to adverse environmental factors. The characteristic seed dormancy and period of germination determine the success or failure of adaptation by plants amongst communities of pathogens and predators (Harper, 1977; Angevine and Chabot, 1979) and relatedly, the seasonal production of flowers often coincide (Tepedino and Stanton, 1981) with emergence of a community of pollen or nectar dependent pollinators or scavengers. The growth and forage yield in Pearl Millet were

significantly affected by the sowing dates (Abd El-Lattief, 2011) and a mid-May cropping regime was recommended for a greater harvest.

The synchronization of two or more mutually dependent communities may also determine the cost of production and success of any cropping season (Oyelana and Ogunwenmo, 2011). Nagananda et al. (2010) reported high germination rate and maximum growth in plantlets of Trigonella foenum graecum from seeds inoculated with 40% concentration of Azobacter and Rhizobium. These micro-organisms and other related ones provide the bio-community and competitive advantage essential for adaptation of this species to its environment.

The period of seed germination, pattern of seedling development, onset of flowers, successful pollination, setting of fruits and production of viable seeds for next generation plants have been ascribed to genetic factors (Holdsworth *et al.*, 2001; Wang *et al.*, 2009). Phenological patterns in plants are often species specific and determine relatedness among community of similar plant species (Ackerly, 2004) and their mode of adaptation and evolutionary history (Pausas *et al.*, 2006). Variation in time of germination and the number of times a species is able to germinate within its growing season or within a year often correlate with its spread and survival.

A considerable difference in the seasonal germination patterns were established for a number of angiosperm species across autumn and through winter to early spring and summer by Brandle *et al.* (2003). Through in vitro screening (Pourhadian and Khajehpour, 2010) the germination time and standard temperature (20°C) required or essential for field emergence of eight wheat strains (Triticum aestivum) was predicted.

Leaf traits including size and pigmentation have been regarded as an adaptation to climate (Ackerly, 2004) and have equally been employed in tracing species ancestral origin or relationship (Oyelana, 2011). Leaf ontogeny including organisation of cells and the subsequent formation of tissues and leaves differentiate few monocot and dicot leaves at primordial stages of growth (Nelson and Langdale, 1989). The remarkable uniformity of organ size, synchronised growth and coordination of response to stimuli (Mian *et al.*, 1998; Mizukami and Fischer, 2000) are intrinsic and under genetic control in most members of angiosperm family.

The differences in leaf and flower size and plant height have been employed as diagnostic tool in tracing relationship among members of *Solanum* (Oyelana, 2011; Knapp, 1991; Edmonds, 1986). However, these features are plastic and unreliable diagnostic tools, as sizes and number are often modified by seasonal climatic variations thereby, making the members of this group so complex to analyse (Omidiji, 1982; Gbile, 1985; Oyelana and Ugborogho, 1997). Organ ontogeny and phenological patterns of leaves (Nelson and Langdale, 1989) and innate germination pattern (El-Kassaby *et al.*, 1992; Baskin and Baskin, 1998; Thompson *et al.*, 1999) have severally been applied in establishing species relatedness among few members of angiosperm (Weigel, 1995; Elliot *et al.*, 1996).

Consequently, we employed the pattern of seed germination and the near fixation of phenological events in seedlings growth to trace and establish relatedness among the eight Solanum species endemic to Nigeria with the hope of elucidating on the evolutionary history and closeness of this group of species.

MATERIALS AND METHODS

Germinability tests: The eight species are as listed on Table 1. Healthy seeds were obtained from the Institute of Agricultural Research and Training and Institute of Horticultural Research, Ibadan, Nigeria and Dr. M.O. Omidiji personal collections. The seeds that completely immersed in

Table 1: Taxonomic classification of the eight Solanum species used for the study

Taxonomic classification	Species name
A. Sub-genus: Leptostemonum (Dun) Bitt.	
I. Section: Melongena dun	
Series Incanifornia Bitt.	
Sub-series euincana bitt.	S. melongena L.
Series macrocarpa dun.	S. macrocarpon L
Series afroindica bitt.	S. anguivi L.
	$S.\ aethiopicum\ { m L}.$
	S. gilo L.
II. Section: Torva nees. Trans. Linn.	
Series eutorvum bitt.	S. torvum SW.
B. Sub-genus: Solanum seithe	
III. Section: Solanum	
Sub-section morella (Dun.) Bitt.	$S.\ scabrum\ L.$
C. Sub-genus: Brevantherum (Seithe) D'Arcy	
IV. Section: Brevantherum	S. erianthum Don

Adapted from Gbile (1985)

water were regarded viable. The 600 viable seeds obtained for each species were sorted into six groups of 50 seeds and used for the different germinability tests. The germinability tests included mechanical and chemical scarification in which the seed testae were punctured at the hilum with the help of a needle pin and seeds pre-soaked in 1% KMnO₄ for 8 h, respectively.

Seeds were pre-soaked in 0.2, 0.4 and 0.6% aqueous solutions of colchicine for 24 and 36 h periods for each regime. Seeds were also pre-soaked in deionised water for 8 h as control experiments. The seeds exposed to 1% $\rm KMnO_4$ and aqueous solutions of colchicine were subsequently rinsed in water and all the treated seeds were later transferred into Petri dishes lined with moist cotton wool and placed in the dark for 3 days at room temperature. Each of the six test groups was carried out in duplicate.

Assessment of germination: The seeds were considered to have germinated when the integument of seeds were ruptured by the radicles. Germination was scored in percentages while the rate of germination was calculated using:

$$\frac{A1+A2.....An}{A1T1+A2T2.....AnTn} \times \ 100$$

which represents the coefficient of velocity of germination where, A is the number of radicles emerged on particular number of days (T) (Ibikunle and Komolafe, 1973).

Cultivation of seedlings: The development of seedlings from the growth of radicles (initial rupture of seed's integument) for emergence of the tenth (10th) leaf was monitored under aseptic conditions in the screen house.

Floral whorls: Four seedlings were cultivated for each of the eight species in the experimental plot of University of Lagos and observed for flower bud initiation and pattern of floral whorls over a 6-month period (two growing seasons).

RESULTS

Structure of seeds: Seeds were ovoid, brown and 3.14 to 3.35 mm in diameter in S. gilo and S. melongena Golden but light yellow in S. macrocarpon (Fig. 1a-b) while they were flattened-reniform, pale yellow and 1.04 to 1.14 mm in diameter in S. scabrum subsp. erectum and 2.12 to 2.48 mm in S. scabrum subsp. scabrum, S. aethiopicum, S. erianthum, S. anguivi and S. torvum.

Germination rate and percentage: Germination was epigeal in all the eight species. Table 2 shows the percentages and rate of germination in the different treatments for the eight species. In the control, germination was 85 and 5%, respectively for S. melongena Golden and S. macrocarpon but ranged between 10 and 50% for the other species. In seeds mechanically scarified, germination (rate) and percentage increased significantly from (0.02) 5% to (0.91) 75%; (0.04) 15% to (0.77) 70% and (0.06) 25% to (0.69) 55% in S. macrocarpon, S. scabrum subsp. erectum and S. aethiopicum, respectively. In chemically scarified seeds germination was (0.09) 85% to (0.11) 90%; (0.12) 45% to (0.25) 55%; (0.06) 25% to (0.13) 60% and (0.05) 45% to (0.10) 65% for S. melongena 'Golden', S. anguivi, S. aethiopicum and S. erianthum, respectively. The rate and percentage germination was not significantly increased in seeds pre-soaked in solutions of colchicine. The rate and percentage germination in seeds exposed to 0.6% colchicine decreased from (0.09) 85% to (0.08) 65%, (0.12) 45% to (0.08) 25% and (0.06) 35% to (0.05) 25% for S. melongena Golden, S. anguivi and S. torvum, respectively.

Table 2: Percentage and mean coefficient of velocity (rate) of germination of seeds

Taxa					Concentration of colchicine and time in hour of pre-soaking					
	a-% germination b-rate of germination	Control 1% KMnO ₄	Mechanical	0.2		0.4		0.6		
			1% KMnO ₄	scarification	24 h	36 h	24 h	36 h	24 h	36 h
S. melongena	a	85	90	100	95	90	95	80	65	55
Golden	b	0.09	0.11	0.89	0.12	0.11	0.12	0.12	0.08	0.12
S. macrocarpon	a	35	40	80	50	40	35	35	25	10
	b	0.09	0.08	0.87	0.36	0.33	0.09	0.06	0.04	0.02
S. anguivi	a	45	55	75	45	40	35	30	25	15
	b	0.12	0.25	0.49	0.10	0.12	0.06	0.06	0.08	0.03
S. aethiopicum	a	25	60	55	45	25	25	35	45	35
	b	0.06	0.13	0.69	0.10	0.05	0.06	0.08	0.10	0.07
S. gilo	a	80	85	95	55	50	65	55	40	35
	b	0.94	0.86	0.96	0.14	0.10	0.10	0.12	0.08	0.04
S. torvum	a	35	45	75	55	50	65	55	25	25
	b	0.06	0.08	0.79	0.10	0.09	0.12	0.10	0.05	0.11
S. scabrum 'scabrum'	a	50	65	90	60	40	55	95	35	35
	b	0.07	0.09	0.87	0.09	0.06	0.08	0.14	0.11	0.09
S. scabrum 'erectum'	a	15	55	70	40	35	35	35	30	5
	b	0.04	0.08	0.77	0.13	0.12	0.11	0.11	0.10	0.02
S. erianthum	a	45	65	85	55	15	15	25	20	10
	b	0.05	0.10	0.96	0.06	0.02	0.04	0.04	0.06	0.02

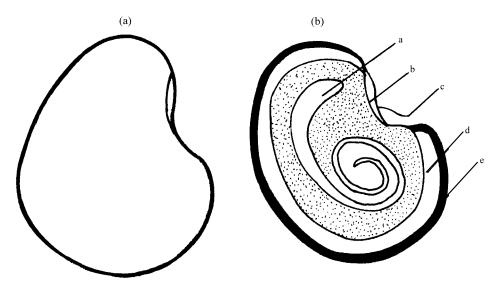


Fig. 1(a-b): Structure of seed, (a) external structure (b) longitudinal section of seed (a-radicle tip, b- endosperm, c-hilum, d-lateral endosperm, e-seed testa)

Germination biology: Germination resumed in seeds 4 days after soaking in all the eight species (Fig. 2a-b). Radicles extended in length and hypocotyl became looped (Fig. 2c) 4th day after protrusion from seeds and seeds emerged from soil (Fig. 2d) by the 6th day. The cotyledons became fully spread (Fig. 2e-f) by the 7th day. The seedlings of *S. scabrum* subsp. *scabrum* had attained the last stage (Fig. 4) by the 5th day. The secondary roots became evident on radicles (Fig. 2d) from 6th day. The growth of radicles in the seeds previously soaked in 1% KMnO₄ showed similar pattern and velocity. However, the growth of radicles from seeds pre-soaked in solutions of colchicine was significantly arrested and about 20% of the emerging seedlings had their cotyledons fully spread by the 12th day. All the radicles from the seeds mechanically scarified dropped and died off three days after germination.

Seedling pattern of growth: The first leaf emerged in bud 10th day of radicle protrusion (Fig. 3a). The first three leaves were elliptic, pinnately veined and margins were entire (Fig. 3a-c) but senescence 48 days afterwards. The fourth leaf emerged in bud 22nd day and was characteristically broader, net veined and variously lobed on the margins (Fig. 3d). The rate of addition of foliages from the 4th leaf was faster in S. aethiopicum and the two subspecies of S. scabrum (Fig. 4). All cotyledons had dropped by the 54 days after germination. Stipules appeared from the 5th leaf and the lateral shoots were initiated from the 54th day after germination. The trichome hairs became prominent on stems of S. scabrum subsp. scabrum from 54th day. However, the first leaf did not appear until the 18th day and the fourth leaf until the 68th day after germination in seedlings from the seeds pre-soaked in solutions of colchicine.

Arrangement of floral whorls: The flowers were bisexual and actinomorphic. A regular flower possesses a wall of 5-7 anthers (stamens) forming a cone shaped structure around the exerted style (Fig. 5a-b) and was placed at obtuse angle to the stem or peduncle of inflorescence (Fig. 5a). In first season shoots, the regular flower types dominated 2/3 portion of the total number of flowers per inflorescence counting from the base while on second season shoots, the number of aberrant flowers

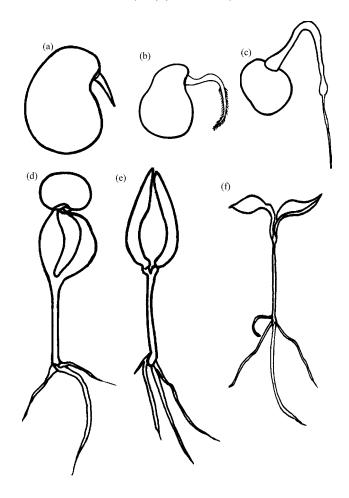


Fig. 2(a-f): Germination Biology-from protrusion of radicle to spread of cotyledons (a) radicle emergence from hilum of seed (b) growth of root hairs (c) looping of hypocotyl, (d) emergence of cotyledons from seed and lifting of seed above soil surface and (e-f) emergence and spread of cotyledons

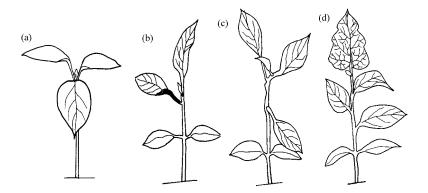


Fig. 3(a-d): Pattern of seedling growth (a) cotyledons and 1st leaf, (b-c) cotyledons and first three leaves (pinnately veined) and (d) the 4th leaf showing net venation and lobbing of margins

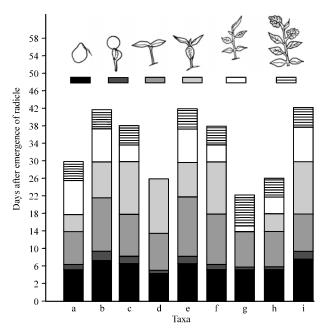


Fig. 4(a-i): Comparative growth rate in the eight species. (a) Solanum melongena Golden, (b) S. macrocarpon, (c) S. anguivi, (d) S. aethiopicum, (e) S. gilo, (f) S. torvum, (g) S. scabrum subsp. erectum (h) S. Scabrum subsp. scabrum and (i) S. erianthum)

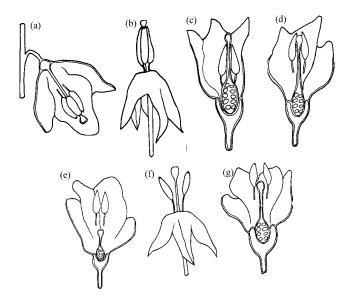


Fig. 5(a-g): Combination of flower types on inflorescence (a) flower placed at obtuse angle to stem, (b) regular flower, (c-d) longitudinal sections of flowers, (e-g) aberrant flowers (e) stigma surface placed beneath stamens, (f) stamens loosely arranged and tips pointing away from stigma surface and (g) stamens projecting slightly above stigma surface

including single sexed (staminate) flowers and those with short style length (Fig. 5c-d) and loose stamens constituted the 2/3 of flowers, counting from the tip of inflorescence. The stigmatic surfaces

were placed beneath anther pores in short style-flowers (Fig. 5e-g). The aberrant flowers were more common on second season shoots of *S. macrocarpon*, *S. aethiopicum* and *S. melongena* Golden than the regular flowers. The numbers of anthers in the staminate flowers ranged between 10 and 14 and were equally visited by insect pollinators.

DISCUSSION

Germination occurred once the seeds testae became permeated with water. Germination took average of 4 days in all the species. Germination was instant in seeds mechanically scarified and the seeds of S. melongena 'Golden' attained 100% germination under 24 h. However, the radicles from these seeds stopped further growth and dropped few days after. Similar innate control of germination was observed in Solanum nigrum (Zhou et al., 2005) and all the seeds mechanical scarified did not break their dormancy. This suggests that Solanum seeds require specific number of days for germination to occur and seedlings to successfully grow to maturity. This requirement varies among the different species of angiosperm and the seeds of Viburnum tinus require over 10 weeks (Karlsson et al., 2005) to break their dormancy and germinate. Koduru et al. (2006) exposed the seeds of Solanum aculeastrum to 100-120°C temperature range for 45-60 min and observed 85% improved germination. Baskin et al. (2006) observed a higher germination and sustained embryo development when mechanically scarified seeds were exposed to appropriate temperatures.

Germination rate and percentage was improved in seeds pre-soaked in 1% KMnO₄. However, the pattern of growth remained similar as described for the control experiment. Germination was induced within two days of treatment of *Lupinus leptophyllus* seeds with sulphuric acid (Alderete-Chavez *et al.*, 2010). Scarification of seeds using cold acid for 20 min followed by 30-40 days stratification in concentrations of 500-1000 mg L⁻¹ gibberellic acid gave the highest percentage germination in Kholkhong seeds (Baninasab and Rahemi, 2008) while a 66.66% germination was achieved for *Quercus ilex* when the seeds were scarified for 1-2 months in cold (5°C) acid (Ghasemi and Khosh-Khui, 2007).

Seed size had no definite effect on rate of germination in all the eight species studied. Similar correlation was reported between seed size and yield in Irish potato (Solanum tuberosum L.) by Babaji et al. (2010). However, Dunlap and Barnett (1983) reported some correlations between seed size and rate of germination in Pinus taeda. The eight Solanum species studied can be classified into four groups based on size and colour of seeds. Seeds were ovoid, brown and 3.14-3.35 mm diameter in S. gilo and S. melongena Golden but yellow in S. macrocarpon while they were flattened-reniform, pale yellow and 1.04-1.14 mm in diameter in S. scabrum subsp. erectum but 2.12-2.48 mm in S. torvum, S. aethiopicum, S. anguivi, S. erianthum and S. scabrum subsp. scabrum. Suthar et al. (2009) described the seeds of Solanum species as slightly discoid, pale yellow or brown with size ranging between 1.04-1.24 mm. Seed size has been found to be under strong genetic influence (Bagchi et al., 1990) and has been employed as tool to trace evolutionary pattern (Baskin and Baskin, 2004; Nikolaeva, 2004) in members of angiosperm family.

The time and sequence of germination and seedling growth followed a fixed pattern in all the eight Solanum species. The sequence of addition and characteristic shapes of foliages were similar and greatly overlapped. The remarkable uniformity in the sequence and developmental patterns help confirm the earlier assertions (Oyelana, 2005; Oyelana and Ogunwenmo, 2005; Suthar et al., 2009) that members of this genus had similar genome. Pinto et al. (2007) reported similar time sequence and pattern of seedling growth for Solanum lycoparcum. They reported a time sequence of 6 and 12 days for radicle protrusion and production of secondary roots respectively while

cotyledons emerged from seeds 9-10 days of radicle growth. However, the profound impact of Molybdenum on the endogenous germination rate, seedling growth and structural organisation of root, shoot and leaves was reported (Datta et al., 2011) for Bengal gram (Cicer arientinum). Similar endogenous rhythm for regeneration of shoots from leaf cuttings was accompanied by the application of growth hormone in Solanum villosum (Hussein and Aqlan, 2011).

Trichome hairs emerged on the stems of *S. scabrum* subsp. *scabrum* almost simultaneously with the senescence of cotyledons and shortly before the initiation of lateral buds. This feature helps separate the two subspecies of *S. scabrum* earlier at seedling stage. The pattern of inheritance of trichome gene in the hybrids from crosses between (*S. phureja* x *S. tuberosum*) x *S. berthaultii* (Mehlenbacher *et al.*, 1983) affirmed its dominant status.

The combination of bisexual and staminate flowers on same inflorescence is of evolutionary significance (Vogler and Kalisz, 2001). The extra anthers on the staminate flowers in some of these species could increase pollen load and ensure greater pollination success. Weekley and Brothers (2006) attributed this to emergence of a mixed mating system in angiosperm. The chances of out crossing of pollen between flowers on inflorescence adjacent, beneath or distantly located could also be enhanced in these species and thereby help to ensure low inbreeding depression in these eight Solanum species.

CONCLUSION

Germination was intrinsic and under genetic control in the eight species. Germination percentage was improved in seeds mechanically and chemically scarified. The pattern of germination and seedling growth were remarkably similar in all the eight species. The size and colour of seeds separated the members of this genus into four groups of ovoid-shaped seeds which may be brown or yellow or flattened-reniform, pale yellow and 1.04-1.14 mm or 2.12-2.48 mm in diameter. The near similar morphological and floral features and the overlaps in the sequence and pattern of seedling development to the 10th leaf reinforce the earlier postulation of a common ancestry for members of this genus. The emergence of trichome hairs on stems of *S. scabrum* subsp. *scabrum* differentiated it from *S. scabrum* subsp. *erectum* early at seedling stages. A regular flower has a characteristic wall of 5-7 anthers arranged in a cone around the exerted style while the aberrant flowers included one or combination of single sexed (staminate) flowers or flowers in which styles were placed beneath the anthers as a result of reduction in length. The regular flowers were about 2/3 of the total number of flowers on inflorescence in the first season shoots while the aberrant flower types dominated the inflorescence of the second season shoots in most of the eight species.

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