

Genetically engineered *Bt* brinjal and the implications for plant biodiversity - revisited

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April 2012

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This report is commissioned by Greenpeace. The views expressed are those of the author alone, and have been developed as a result of critical evaluation of all the information available at the time of writing. Responsibility for the content of this report and any faults contained therein lies with the author.

Abbreviations used in this report:

Bt: *Bacillus thuringiensis*

CBD: Convention on Biological Diversity.

DAP: Department of Agriculture of the Philippines.

FAO: Food and Agriculture Organisation.

GEAC: Genetic Engineering Approval Committee, Ministry of Environment and Forests, Government of India.

MOEF: Ministry of Environment and Forests, Government of India.

Glossary

allogamy: cross-fertilization brought about by fusion of male and female gametes from genetically dissimilar individuals of the same species.

anthers: the upper portion of the stamen, from where pollen is released.

anthesis: the period during which a flower is fully open and functional.

apical: the region at the tip of an anther or shoot.

brachystyl: (in flowers) having shortened styles.

dehiscence: opening of part of the anther to release pollen.

feral: (in plants) existing as a result of a change from being cultivated to being uncultivated, wild or weedy.

hermaphrodite: (in flowers) having both male and female reproductive parts in the same structure.

heterosis: the exhibition by a hybrid of more vigorous growth, greater yield or increased disease resistance.

hybrid: an individual derived from genetically dissimilar parents.

hybridization: processes that lead to the formation of a hybrid.

inbreeding: the production of offspring by the fusion of genetically closely-related gametes, eg. by self-fertilization.

indumentum: a covering of hairs.

inflorescence: a group of flowers.

introgression: the incorporation of genes from one species into another.

landrace: an ancient or primitive cultivar of a crop plant.

open-pollinated: unaided pollination that has been allowed to take place without any barriers.

outbreeding: the production of offspring by the fusion of genetically distantly-related gametes, eg. by cross-fertilization.

stellate hairs: hairs with star-like branches, forming part of the indumentum.

sympatric: occupying the same geographic region.

transgene: a gene that has been transferred by genetic engineering techniques from one organism to another.

Summary

Concerns over the commercial release of genetically engineered (GE), also called genetically modified (GM), *Bt* brinjal, also called aubergine or talong, relate to biosafety. One major concern is the potential for transgene flow from *Bt* brinjal to wild, weedy and cultivated relatives. As a consequence, selective advantage gained by the *Bt* pest resistance gene could enable these relatives to become aggressive weeds. The potential for disruption of ecological balance and plant biodiversity is therefore considerable.

Our knowledge of the diversity and taxonomy of species related to the brinjal eggplant in South and South-East Asia is limited. This includes areas where the *Bt* eggplant is being considered for cultivation: India, Philippines and Bangladesh. Difficulties over identification and nomenclature of brinjal relatives have caused problems with the interpretation of experimental data. There is thus a prime need to develop our understanding of the systematics of this group. A brief summary of the taxonomy and characteristics of brinjal and its closest wild relatives (part of the brinjal eggplant complex) is given, to help lessen the ongoing confusion over the names, distribution and agronomic features of these closely-related taxa.

Successful hybridization between untransformed brinjal and at least 10 species in *Solanum* subgenus *Leptostemonum* found in India and South-East Asia can be demonstrated, as can hybridization between *Bt* brinjal and one wild species. Crossing *Bt* brinjal and untransformed brinjal can also produce hybrids. There is a need to test several other species in subgenus *Leptostemonum* for crossability with both untransformed and transformed brinjal. In such future studies, standardization of experimental methodology would enable consistent interpretation of results.

The breeding system in brinjal is a combination of both inbreeding and outbreeding, with insect-pollination featuring strongly in outbreeding. These factors should be re-assessed when considering the outcrossing potential of *Bt* brinjal and isolation distances in field trials.

Studies of gene flow and weediness in *Bt* brinjal that have been performed to date are limited, both in number and content. Fertility in brinjal is typical of the spiny group of solanums, and the implications for increased weediness of both *Bt* brinjal itself, as well as hybrids carrying the *Bt* transgene are significant. As these are crucial aspects of environmental risk assessment of the effects on plant biodiversity, detailed, long-term studies are required to determine the extent to which these are likely to occur.

In the context of Convention on Biological Diversity biosafety and biodiversity guidance, there is an urgent need for more detailed studies on hybridization, gene flow, weediness and systematics of brinjal and its wild, weedy and cultivated relatives. The implications for plant biodiversity of the outdoor cultivation of GE *Bt* brinjal eggplant, including field trials and commercial growing, cannot be fully assessed in their absence.

I. Introduction

The edible eggplants

Across the world there are around 25 cultivated food species of *Solanum*, a genus of the nightshade family, or *Solanaceae*, which includes the potato, tomato and various eggplants (Samuels, 2009). *S. melongena* L. is generally known as the brinjal eggplant (Lester & Daunay, 2003) and talong in the Philippines. It is a popular vegetable throughout Asia. The latest annual crop production figures for this region (in excess of 37m metric tonnes, with a market value of around \$8bn US dollars; FAO, 2011) reflect this. Other, closely related eggplant species are *S. aethiopicum* L. (scarlet eggplant) and *S. macrocarpon* L. (Gboma eggplant) widely cultivated in Africa, and *S. torvum* Sw. (pea eggplant) and *S. lasiocarpum* Dunal (hairy eggplant) cultivated in Asia.

The brinjal eggplant is a shrubby, herbaceous or semi-woody plant with a fibrous root system. It grows to a height of 0.5 to 2.0m with an erect or spreading, determinate growth habit. It is a perennial in warmer regions, but cultivated as an annual in temperate regions. Anthocyanin pigmentation, prickles and hairiness on vegetative parts vary widely. The leaves are alternate, simple, ovate, lobed, and more or less covered with stellate hairs. Inflorescences have one to several flowers, with various degrees of andromonoecy (proportion of functionally male flowers to hermaphrodite flowers) shown by different cultivars. Many commercial forms display solitary hermaphrodite flowers. Flowers are violet to purple and generally 5-merous (5 sepals, 5 petals, 5 stamens), but 6, 7 and 8-lobed flowers are common in modern cultivars.

The fruit is a pendent, fleshy berry, ranging in colour from green, white, yellow, pink, violet to dark purple, with varying degrees of mottling or striping on the skin. The shape ranges from spherical, oval, oblong to cylindrical. Fruit size may vary anywhere between 4 to 45cm long and between 3 and 12cm in diameter, with a weight range of 15 to 1500g. It is eaten when immature and usually cooked, although in some South-East Asian countries may be eaten raw.

Crop losses in brinjal

Brinjal is susceptible to many diseases and pests, including the eggplant fruit and shoot borer (EFSB, *Leucinodes orbonalis* Guenée). EFSB is a medium-sized moth (*Lepidoptera: Crambidae*) whose feeding larvae cause damage to the brinjal crop by boring into stems and fruits. Yield, therefore, can be affected either by severely damaged or destroyed fruits, or by damage to the developing plant. EFSB has been described as the most serious and destructive pest to brinjal crops and may cause up to 90% losses in yield (Parimi & Zehr, 2009). The Report of the Expert Committee (EC-II) on *Bt* Brinjal submitted to the Indian government (GEAC, 2009) described losses of between 60-70%, even when insecticides were used. A review of brinjal losses to EFSB in India was given by Andow (2010) and showed that, in fact, losses may be generally over-estimated. EFSB damage in India lies anywhere between 5.3-54.8% of the total yield of crop. Losses vary considerably between the various Indian states and between climatic seasons, but seem to average out at around 30% (Andow, 2010).

GE *Bt* brinjal and pest resistance

The chromosome number of *S. melongena* is $2n=24$, with a genome size of approximately 956Mb. This is estimated to code for 35-40,000 genes, similar to potato and tomato, in addition to possessing repeat elements representing a major part of the genome (Frary et al, 2007). Recently, an insect-resistant hybrid, 'EE-1 *Bt* brinjal', has been developed through genetic engineering (also known as genetic modification). 'Event EE-1' is genetically engineered (GE) to express a crystalline (Cry) protein toxin similar to that produced naturally by the soil bacterium *Bacillus thuringiensis* Berliner ('*Bt*'). EE-1 is brought about by the insertion of a chimeric (fused) *Bt* gene Cry1Ab-Cry1Ac inserted into the genome, remaining 'switched on' and effective in all parts (Seralini, 2009). The modified *Bt* toxin has been developed to endow pest-resistance to lepidopteran pests, such as EFSB. It is lethally toxic and works via binding to protein receptors in the gut of the larval stage of the pest, whilst it feeds on the modified plants.

Bt brinjal has undergone field evaluation by a national hybrid seed company in India and further progress into backcrossing Event EE-1 into locally adapted, open-pollinated brinjal varieties has recently been made in the public sector (MOEF, 2010). The technology has also been passed on to the public sector in the Philippines, where multilocation field trials are in progress (IPB, 2011), as well as both public and private sectors in Bangladesh (MOEF, 2010). Biosafety trials and field evaluation are also planned in India for other versions of *Bt* brinjal using Cry1Fa1 and Cry1Ab genes (MOEF, 2010).

The *Bt* brinjal debate

There is an on-going debate on the need for the commercialization of *Bt* brinjal. Proponents of the GE crop argue that widespread adoption of the *Bt* form will enable a sizeable reduction in the use of insecticides, thus making the growing environment less harmful for invertebrates and other animals. Along with this, a substantial increase in crop yields and greater food availability can be expected. Alongside food safety concerns, opponents argue that there is a potential for toxic effects on populations of non-target invertebrates, and for consequent knock-on effects on other animal groups. Farmers may also move towards the cultivation of a restricted number of GE forms, potentially replacing traditional landraces. Alongside these issues there is the additional concern over the transfer of the *Bt* transgenes to non-GE brinjal or its wild relatives, and the consequences for plant biodiversity.

2. Gene flow from transgenic crops to crop wild relatives

One of the hazards arising from the introduction of GE crops into the environment is that associated with the integration and subsequent expression of the transgene (the genetic construct inserted into the GE plant genome) in a different species (Craig et al, 2008), as well as gene flow to non-GE forms of the crops.

Since the 1980s, the realization that transgenes could move from cultivated crops to populations of their wild relatives has brought attention to the hitherto neglected area of crop-to-wild gene flow (Ellstrand, 2003). Since the advent of GE crops such as *Bt* brinjal, GE cotton, maize, rice and soya into the environment, crop-to-wild gene flow research has grown (eg. Craig et al, 2008; Ellstrand, 2003; Ellstrand et al, 1999; Jenczewski et al, 2003; Lu, 2003; Martinez-Castillo et al, 2007; Papa & Gepts, 2004; Singh et al, 2006; Snow, 2002).

One potential problem associated with crop-to-wild hybridization is the evolution of increased weediness or invasiveness in wild relatives (Dale et al, 2002). In general, first generation hybrids are more similar to the wild than to the domesticated forms and, along with the possibility of heterosis, the first generation hybrids will have a much higher reproductive success in the wild than in the domesticated environment (Papa & Gepts, 2004). In subsequent generations, both selection against domesticated alleles and asymmetric gene flow will favour introgression of genes from domesticated to wild populations, rather than in the opposite direction (Papa & Gepts, 2004).

Similarly, in the case of transgene escape, gene flow will occur from GE crops to their uncultivated, weedy or wild relatives unavoidably (Ellstrand et al, 1999; Snow, 2002). Unless such transgenes are deleterious, they will generally persist and introgress into the natural populations (Ellstrand, 2003).

Additionally, if a transgenic crop has a sympatric distribution, synchronized flowering period and is sexually compatible with its weedy or wild relatives, introgression of transgenes into the wild species will occur at a considerable frequency (Dale, 1994; Ellstrand & Hoffman, 1990). Furthermore, introgression of pest resistance transgenes, such as that carried by *Bt* brinjal, will confer a selective advantage which might encourage unnaturally aggressive growth. Thus, the potential to disrupt ecological balance and biodiversity in a relatively short time is considerable. For this reason, risk management of GE plants may make it necessary to monitor significant wild and weedy populations that might be affected by transgene escape (Singh et al, 2006).

3. The centre of origin of brinjal

The probability of crop-to-wild gene flow depends on the geographic distribution of crops and their wild relatives (Jenczewski et al, 2003). In this context, caution has been advocated (eg. SCBD, 2000) over the release of GE crops into their centres of origin ie. the geographical regions in which they were domesticated and where wild relatives and/or weedy forms persist. Of Vavilov's (1951) eight 'centres of origin' of crop plants, India forms a major part of the Indo-Burma Centre (which also includes Assam and Myanmar), the region in which crops such as brinjal eggplant are presumed to have been domesticated. Most recent authors (eg. Daunay et al, 2001a) also subscribe to this view. In line with this theory, *S. melongena* is believed to have originated in the Indian subcontinent (Arora, 1991; MOEF, 2010; Chen & Li, 1996; Isshiki et al, 1994; Samuels, 2010a; Swarup, 1995; Zeven & Zhukovsky, 1975). Recent archaeological evidence (Kashyap, 2006; Kashyap & Weber, 2010) supports initial domestication of brinjal in north-west India, pre-dating the earliest record of cultivation in East Asia of 59 BC (Wang et al, 2008) by around 2 000 years.

Various other candidate regions for the domestication of *S. melongena* have been proposed by other researchers, including Africa (Sampson, 1936), India/south-east China (Doganlar et al, 2002), Burma to Indo-China (Daunay et al, 2001b) and South-East Asia (Lester & Hasan, 1991). As a consequence of the varying views on where untransformed brinjal was domesticated, a centre of origin debate has arisen (Bhargava, 2010; CSA, 2006; Hanur, 2011; Kameswara Rao et al, 2011; Samuels, 2011a, 2011b). However, in the context of the *Bt* brinjal controversy, the importance of the centre of origin is not so much its precise location, as the contention that brinjal wild relatives can be expected to occur there (Samuels, 2011b).

The 'centre of diversity' may or may not occupy the same location as the centre of origin, as crops which were originally domesticated in a certain region may, due to human migration, etc, have been transferred elsewhere. In the subsequent location, intensive human selection, along with further spatial isolation, would then encourage the increase in genetic diversity of the crop over many years. According to Daunay et al. (2001b) the whole of South-East Asia, from India to China and Indonesia (including the Philippines), is the primary diversification centre for brinjal eggplant, and the greatest morphological diversity (ie. range of landraces and cultivars) is found there. Within this region, Hawkes (1983) pinpointed India as the centre of diversity for brinjal. There is thus a vast wealth of brinjal plant genetic resources to be found in India. Many regions and districts have a wide variety of traditional landraces, eg. Mattu Gulla, from Karnataka, south-west India (Bhat & Vasanthi, 2008), as well as a multitude of nationally popular varieties. The National Gene Bank (National Bureau of Plant Genetic Resources, New Delhi) holds 2 782 landraces of brinjal, as well as 530 'wild and weedy' accessions (Sharma et al, 2010) from all over the subcontinent. Other countries of South-East Asia have diverse collections of *S. melongena*, eg. the National Plant Genetic Resources Laboratory of the Philippines, which holds around 500 local accessions (DAP, 1995).

4. Wild, weedy & cultivated relatives in South and South-East Asia

Floristic treatments

Recent floristic treatments of *Solanum* for many parts of India (and other parts of South and South-East Asia) are scarce. There remain widely-differing views on how many species there are, and several under-estimates ranging between 15 and 26 species have been published (Andow, 2010; Chaudhary & Gaur, 2009; MOEF, 2010). More recently, Kameswara Rao (2011) in his summary based on the Botanical Survey of India, lists 48 species of *Solanum* in India. Apart from the outline of common synonyms noted by him, at least one more species, *S. nightii* Nees, can be further synonymized to *S. pubescens* Willd. (Lester et al, 1999). It is also likely that there are several other, as yet unrecorded, cultivated exotics which may or may not yet have been naturalized. A realistic round figure of 50 species is suggested here, 36 of which are members of subgenus *Leptostemonum* (Dunal) Bitter, the ‘spiny solanums’ (Table 1).

This spiny *Solanum* group includes the brinjal eggplant and its wild relatives, as well as allied, prickly, adventive species of the tropics and sub-tropics. Many of these are naturally occurring in India, but some have been introduced as cultivated crops or ornamental species (largely from Africa, Central and South America). Subgenus *Leptostemonum* contains widely variable species which are notoriously difficult to identify accurately. In addition, many of the species names have complex synonymies (see, for example, Andow, 2010), whereby they have been known by several different names, sometimes over a period of many years. For example, there are over 50 synonyms for *S. melongena* alone, some of which go back to the 18th century! This has led to a difficult taxonomic situation for this group in India and researchers who are without a sound knowledge of the taxonomic intricacies of this group therefore find themselves at a disadvantage.

Table 1. *Solanum* Subgenus *Leptostemonum*: wild, cultivated and naturalized species found in India. (Partly based on Daunay et al, 1991; Jaeger, 1985; Kameswara Rao, 2011; Nee, 1999). For reasons of space, synonyms are not given here; for detailed synonymies see: Kameswara Rao, 2011; Solanaceae Source- <http://www.nhm.ac.uk/research-curation/research/projects/solanaceaesource/project/>)

| Section <i>Acanthophora</i> Dunal | Section <i>Melongena</i> (Miller) Dunal | Section <i>Oliganthes</i> (Dunal) Bitter |
|---|---|--|
| <i>S. aculeatissimum</i> Jacq. [!] | <i>S. cumingii</i> Dunal! [*] | <i>S. aethiopicum</i> L. ^{!*} |
| <i>S. capsicoides</i> All. | <i>S. elaeagnifolium</i> Cav. | <i>S. cordatum</i> Forsskal |
| <i>S. mammosum</i> L. | <i>S. heteracanthum</i> Dunal | <i>S. forskalii</i> Dunal [!] |
| <i>S. viarum</i> Dunal [!] | <i>S. incanum</i> L. ^{!*} | <i>S. hovei</i> Dunal |
| Section <i>Anisantherum</i> Bitter | <i>S. insanum</i> L. ^{!*} | <i>S. kurzii</i> Prain [!] |
| <i>S. pubescens</i> Willd. [!] | <i>S. macrocarpon</i> L. ^{!*} | <i>S. trilobatum</i> L. [!] |
| Section <i>Cryptocarpum</i> Dunal | <i>S. marginatum</i> L.f. ^{!*} | <i>S. violaceum</i> Ortega! [*] |
| <i>S. sisymbriifolium</i> Lam. [!] | <i>S. melongena</i> L. ^{!*} | Section <i>Torva</i> Nees |
| Section <i>Erythrotichum</i> (Whalen) Child | <i>S. ovigerum</i> Dunal! [*] | <i>S. donianum</i> Walp. [!] |
| <i>S. robustum</i> H. Wendl. | <i>S. rostratum</i> Dunal | <i>S. barbisetum</i> Nees |
| Section <i>Herposolanum</i> Bitter | <i>S. virginianum</i> L. ^{!*} | <i>S. giganteum</i> Jacq. [!] |
| <i>S. wenlandii</i> Hook. f. | <i>S. wrightii</i> Benth. [!] | <i>S. hispidum</i> Pers. ^{!?} |
| Section <i>Ischyraanthum</i> Bitter | Section <i>Monodolichopus</i> Bitter | <i>S. sarmentosum</i> Nees |
| <i>S. arundo</i> Mattei | | <i>S. torvum</i> Sw. ^{!*} |
| Section <i>Lasiocarpa</i> (Dunal) D’Arcy | <i>S. coagulans</i> Forsskal [!] | |
| <i>S. lasiocarpum</i> Dunal | | |

[!] = species tested for crossability with *S. melongena*

^{*} = F1 hybrids with measurable pollen stainability produced as a result of sexual crosses

[?] = F1 hybrids produced/pollen stainability not recorded

Adventives and semi-cultivation

Several of the spiny *Solanum* species listed in Table 1, such as *S. aculeatissimum*, *S. capsicoides*, *S. cumingii* (see Figure 1), *S. donianum*, *S. lasiocarpum*, *S. torrum* and *S. viarum*, are found right across tropical South and South-East Asia (including India, Bangladesh and the Philippines) as widespread weeds, or naturalized or cultivated exotics. Because of their adventive nature, they can adapt rapidly to colonizing disturbed ground along roadsides, amongst crops in small-scale cultivations and on the edges of villages. For example, cultivated crops such as *S. aethiopicum* and *S. macrocarpon* (originally from Africa) are found naturalized in the Philippines (DAP, 1995) and elsewhere. Conversely, many wild species which produce fruits with food or medicinal value are often semi-cultivated and encouraged to grow in the vicinities of settlements. Examples of these are *S. barbisetum*, *S. cumingii*, *S. kurzii*, *S. lasiocarpum* and *S. viarum*, used for food, *S. cordatum*, *S. incanum* and *S. violaceum*, used in traditional medicine, and *S. virginianum*, used for both.

Endemics

A considerable number of endemic spiny *Solanum* species are found in various parts of South-East Asia, for example *S. lianoides* Elmer (Elmer, 1910), *S. luzoniense* Merrill (Merrill, 1918), *S. retrorsum* Elmer (Elmer, 1908) and *S. sparsiflorum* Elmer (Elmer, 1913) - all collected in the Philippines, and *S. thorelii* Bonati (Bonati, 1913) - collected in Vietnam. Although originally named as new species in the first quarter of the 20th century, their distributions, taxonomic affinities and conservation status have yet to be studied. A similar lack of knowledge of many other related species is evident. Furthermore, new species of *Solanum* are regularly being collected from tropical Asia, eg. *S. camranbense* Dy Phon & Hul and *S. sakbani* Hul (Hul, in press). The inventory of species closely related to brinjal will undoubtedly expand as more collecting projects are realized.



5. Taxonomic challenges

S. melongena and its close relatives

The taxonomy of species related to the eggplant remains a challenge (Daunay, 2008) and several groups are well-known for being taxonomically elusive. In particular, many wild relatives of brinjal have caused taxonomic difficulty since the earliest collections in the 1800s, and the type specimens of several pivotal taxa have yet to be typified formally. The highly variable nature of this particular group has caused many problems with identification, and species delimitations have been difficult to establish. Some wild taxa show a developmental transition from having robust, strongly armed, juvenile shoots with dense indumentum, to having smaller, less prickly, mature shoots with finer indumentum; juvenile and mature leaves may also differ considerably in lamina size and lobing (Chiarini et al, 2007). There is also considerable interfertility between brinjal and its wild relatives, and amongst the wild relatives themselves (Jaeger, 1985; Lester & Daunay, 2003; Lester & Hasan, 1990, 1991; Pearce & Lester, 1979; Samuels, 2010b, in press). Certain taxa are able to hybridize, leading to intermediate forms. Historically, both in herbaria and in the field, *S. melongena* has been confused with the closely related *S. incanum*, as well as *S. violaceum*, *S. virginianum*, *S. torvum* and other, more distantly related taxa. The taxonomy of brinjal and its wild and weedy relatives has consequently challenged many authors (eg. Deb, 1989; Furini & Wunder, 2004; Karihaloo & Rai, 1995; Lester & Daunay, 2003; Lester & Hasan, 1990, 1991; Mace et al, 1999; Weese & Bohs, 2010).

In several recent taxonomic papers relating to the brinjal eggplant (eg. Daunay & al., 2001a; Weese & Bohs, 2010) much of the salient information available to the authors was based on data (Lester & Hasan, 1991) originally published 20 years ago. As species concepts for members of this group have become broader (Samuels, 2010a), thus the details of descriptive taxonomy, interfertility and distribution have changed over the years. Much of the research focus on hybridization between *S. melongena* and its wild relatives has been on this group; it is therefore important to be clear on their precise identities.

The brinjal eggplant complex

The brinjal eggplant, together with its closest wild and weedy relatives in Asia (*S. melongena sensu lato*), and those in Africa, the Middle East and West Asia (*S. incanum sensu lato*), comprise the 'brinjal eggplant complex' (Samuels, 2010a). The taxa comprising *S. incanum s.l.* are classified as three species: *S. campylacanthum* A. Rich., *S. incanum* L. and *S. lichtensteinii* Willd. (Samuels, 2010b). Furthermore, *S. incanum* L. *sensu stricto* is found in parts of Africa and the Middle East, as well as north-west India (Samuels, in press). It is believed to be the wild ancestor of *S. melongena* (Daunay & al., 2001a; Lester & Hasan, 1991). The various taxa assembled under *S. melongena s.l.* represent a transitional series ranging from weedy and wild forms, provisionally named '*S. insanum* L.' and '*S. cumingii* Dunal', to localized landraces, provisionally named '*S. ovigerum* Dunal', to commercial cultivars, *S. melongena* L. *sensu stricto*. There have been many difficulties over the distinction between *S. incanum* and *S. insanum* (not least of all because of the similarity of the names) on the one hand, and between *S. insanum* and *S. cumingii* on the other. In addition, there are many differing views on the synonymy of taxa such as *S. cumingii*, *S. undatum* Lam. and *S. trongum* Poir., found in South-East Asia (see, for example, Samuels, 2010a). In the absence of a modern review the information in Table 2 below provides a summary of the important characteristics and commonly used names of the South and South-East Asian species in the brinjal eggplant complex.

Table 2. Characteristics of the South and South-East Asian species of the brinjal eggplant complex. (Based on: Daunay et al., 2001a; Hasan, 1989; Karihaloo & Rai, 1995; J Karihaloo, pers. comm; Lester & Hasan, 1990, 1991; Samuels, 2010b, in press; Samuels & Lester, unpubl. Group designations follow Lester & Hasan, 1991).

| Name | Distinguishing characteristics | Distribution |
|---|---|---|
| <i>S. incanum</i> L. <i>sensu stricto</i> (sensu Samuels, 2010b)/ <i>S. incanum</i> Group C/‘bitter tomato’ | <ul style="list-style-type: none"> -compact shrubs, up to 2m -strongly armed, mostly curved prickles -leaves broadly ovate, subentire to moderately lobed, densely tomentose -inflorescence with up to 15 flowers, max. of 3 ♀ -flowers violet to purple -fruits globose, 3.0-3.5cm diameter., bitter | -wild, undisturbed habitats, and semi-cultivated; centred in northeast Africa, extending to western Africa, and to northern India |
| <i>S. insanum</i> L. (sensu Daunay et al, 2001a)/ <i>S. melongena</i> L. var <i>insana</i> (L.) Prain (sensu Lester & Hasan, 1991)/ <i>S. melongena</i> Group E/‘mad apple’ | <ul style="list-style-type: none"> -sprawling shrubs, up to 0.5m high -strongly armed, straight or slightly curved prickles -leaves broadly lanceolate to ovate, shallowly lobed, tomentose -inflorescence with between 5-9 flowers, max. of 3 ♀ -flowers violet -fruits globose or slightly ovoid, 3.0-4.0cm diameter, bitter | -weedy and semi-wild; disturbed habitats, Pakistan, northern and central India, Sri Lanka |
| <i>S. cumingii</i> Dunal (sensu Daunay et al, 2001a)/ <i>S. melongena</i> Group F/‘wild brinjal’ | <ul style="list-style-type: none"> -shrubs, up to 1m high -moderately armed, prickles more or less straight -leaves broadly lanceolate to ovate, moderately lobed, more or less pubescent -inflorescence with up to 3 flowers, 1 ♀ -flowers violet -fruits globose or slightly ovoid, 2.5-3.0cm diameter, bitter | -weedy and semi-wild; disturbed habitats, eastern India, southeast Asia, Philippines and southern China |
| <i>S. ovigerum</i> Dunal (sensu Daunay et al, 2001a)/ <i>S. melongena</i> Group G-primitive cultivars/brinjal landraces | <ul style="list-style-type: none"> -shrubs, up to 1m high -armed or unarmed -leaves broadly lanceolate to ovate, subentire to moderately lobed, more or less pubescent -inflorescence with up to 4 flowers, max. of 3 ♀ -flowers violet -fruits globose, ovoid or oblong, 3.0-4.0cm diameter, mostly non-bitter | -localized landraces; grown in gardens, South Asia, South-East Asia, Philippines |
| <i>S. melongena</i> L. <i>sensu stricto</i> /Group H- advanced cultivars/common brinjal | <ul style="list-style-type: none"> -compact annual herbs, up to 1m high -prickles rare, if present found on calyx and occasionally elsewhere -leaves broadly lanceolate to ovate, subentire to moderately lobed, more or less pubescent -inflorescence with up to several flowers, only 1 ♀ in many cultivars -flowers violet to purple -fruits globose, ovoid, cylindrical, pear-shaped, snake-like, etc; skin colour purple, white, yellow, etc, up to 12cm diameter and up to 30cm or more long, mostly non-bitter | -commercial cultivars; cultivated worldwide |

6. The potential for *Bt* brinjal to hybridize

Breeding system

The structure of the apical pores of the anthers of brinjal flowers tends to direct discharged pollen away from the stigma, thus reducing the likelihood of self-pollination (Quagliotti, 1979). The extent to which self-pollination occurs in some cultivars has been recorded as 0.7-15% (Sambandam, 1964). Brinjal eggplant is 'an often cross-pollinated crop' (Singh, 2009) and Daunay et al (2001a) described it as having a highly variable tendency towards allogamy (up to 70%), according to the presence or absence of pollinating insects and climatic conditions. Swarup (1995) estimated cross-pollination at up to 20%, whilst Quagliotti (1979) gave a range of 0.2-46.8%. MOEF (2010) forwarded regional values of 0-48% for India, 3-7% for China, and 0-8.2% for Taiwan (Asian Vegetable Research Development Centre). The data for India are in need of an update, as the studies that generated them were performed between the 1930s and 1980 (MOEF, 2010). Some of the studies used examination of F1 seedlings to determine cross-pollination levels. Because of the erratic nature of fruit set and seed germination in some cultivars of *S. melongena*, direct techniques such as fluorescence microscopy of gynoecial parts (eg. Sekara & Bieniasz, 2008) would give a more realistic idea of outcrossing levels. Generally, then, the breeding system in brinjal is a combination of both inbreeding and outbreeding.

Flower forms

The flowers of modern cultivars of brinjal eggplant are often solitary and hermaphrodite. Traditional cultivars and landraces may display flowers borne in cymose inflorescences (groups of flowers whose terminal points have limited growth) in which the lower flower is hermaphrodite and the several other, distally arranged flowers are functionally male. This is a trait known as andromonoecy, and is common in subgenus *Leptostemonum*. Heterostyly (technically, andromonoecy with brachystyly) leads to variation in the size of the style and stigma, along with the size of the ovary, and is a trait associated with outbreeding. In brinjal, four flower types (long-styled with large ovary, medium-styled with medium ovary, pseudoshort-styled with rudimentary ovary, short-styled with rudimentary ovary) have been reported (Krishnamurthi & Subramaniam, 1954). Alternatively, Quagliotti (1979) and Sekara & Bieniasz (2008) recognize three types of flower with short, medium or

long styles. The lengths of the stamens in each flower form do not vary. The ratio of the flower types varies with the cultivar and the stage of development (Chen & Li, 1997). Long-styled forms have their style and stigma projecting beyond the cone of anthers (Fig. 2) and are thus much more likely to be cross-pollinated, whilst the medium-styled form is more likely to be self-pollinated. There is a close relationship between stigma position and fruit set (Pradeepa, 2002) and, as a rule, long- and medium-styled flowers invariably produce fruits. Fruit set in long-styled (including solitary) flowers varies from 70-87%, and that of medium-styled between 12.5-55.6% (MOEF, 2010). Opinions vary as to fruit set in short-styled flowers (Fig. 3); some authors (eg. Krishnamurthi & Subraminam, 1954; Pradeepa, 2002) claim they do not produce fruit, but more recent studies (eg. Sekara & Bieniasz, 2008) have demonstrated that they do.

Pollination

In brinjal, 30-40% of fruit set depends on pollination by contact, gravity and wind, and the remainder by insects; many trials indicate that insects play a major role (Quagliotti, 1979). Some pollen leaves the apical pores of the anthers of its own accord upon dehiscence, but physical contact is known to facilitate the exit of pollen. However, some reports have surprisingly described the role of insects in pollination of *Solanum* flowers as 'insignificant' (eg. GEAC, 2007). Cross-pollination of brinjal in Asia is by insects such as bumblebees, (*Bombus* spp.), wild bees (*Exomalopsis*, *Xylocopa*, *Anthophora* spp.) and domestic bees (*Apis* spp.) (MOEF, 2010). Pollen is collected and transferred by some bee species (eg. *Bombus* spp.) using 'buzz-pollination' (Buchman, 1983), by which release of pollen from the anther pores of one flower is encouraged by vibrations set up by rapid beating of the bee's wings. It is then transferred to the stigmas of other flowers as the bee moves on.

Cross-pollination was a significant factor in two sets of gene flow studies, which investigated the transfer of genes from *Bt* brinjal to untransformed brinjal. These were conducted in 2002-2003 and 2009 at locations in India (Chaudhary & Gaur, 2009). Interestingly, bees were employed to assist pollination in this study (Singh, 2009). Results show that outcrossing (indicated by the presence of the *Bt* gene in F1 progeny) was measurable: 1.46-2.7% in the first study and 0.14-0.85% in the second. Furthermore, even relatively low gene flow

rates such as those obtained could be biologically significant (Andow, 2010), particularly if the genetic trait is advantageous for the recipient population and/or the trait is recurrently introduced (Haygood et al, 2003). *Bt* pollen was shown to be transferred away from the transformed plants-up to 20m in the first study and up to 30m in the second-presumably by bees! However, it is likely that this ‘pollen flow’ distance was underestimated – this is due to erroneous experimental design, such as arrangement of the experimental plots and the use of honey-bees (Andow, 2010). Native, buzz-pollinating bees would be more efficient pollinator species. Chaudhary & Gaur (2009) correctly state that *Solanum* pollen is sticky (an adaptation which facilitates insect-pollination), and add that it cannot travel large distances if airborne. An isolation distance of 300 m was recommended for field trials of *Bt* brinjal in India (GEAC, 2009). However if pollen becomes insect-borne (eg. attached to visiting wild insects) kilometres, rather than metres may be covered, after which pollination may be effected elsewhere.



Figure 2. *S. melongena* ‘Black Beauty’ cultivar: long-styled form.

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Crossability of *S. melongena* with other spiny solanums

Although records of natural (as opposed to experimental) inter-specific hybridization between wild species and cultivated brinjal (eg. Viswanathan, 1975) are rare, the number of confirmed reports are probably not indicative of the situation in nature. However, numerous investigations employing experimental cross-pollination between brinjal and its wild relatives have been undertaken, albeit some time ago. Although successful crosses in these studies were produced artificially, they are an indication of what may occur in nature, where brinjal cultivation takes place near to these other species. Several summaries of much of the available data on crossability have been published (eg. Daunay et al, 1991; Kashyap et al, 2003).



Figure 3. *S. melongena* ‘Tres Hative de Barbantane’ cultivar: short-styled form.

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Hybridization between *Bt* brinjal and untransformed species

Pollen flow studies (Singh, 2009) demonstrated that *Bt* brinjal and untransformed brinjal will successfully hybridize (with success taken to be the production of fruits with viable seeds leading to production of an F1 generation). The pollen stainability (a measure of the fertility of the hybrids) was not recorded, however. *Bt* brinjal is 'easily crossable' with *S. incanum* (ascertained by the production of fruit with viable seed) using selected *Bt* brinjal hybrids (IIVR, 2008). This, combined with the relatively high level of interfertility between the two species (Lester & Hasan, 1991), and the tendency for crosses to be more successful if *S. incanum* is the female parent (Baksh & Iqbal, 1979; Khan & Rao, 1976) mean that there is a likelihood for transgene transfer to *S. incanum* from *Bt* brinjal.

Summary of Crossability

Table 1 shows the South and South-East Asian spiny *Solanum* species that have so far been tested for crossability with brinjal, indicating those that successfully produce viable F1 hybrids with stainable pollen. The information shows that 10, possibly 11, species hybridize with *S. melongena*. These species belong to three sections (*Melongena*, *Oliganthes* and *Torva*); not surprisingly, most belong to *Melongena*. It is worth adding that the fertility links between species are noticeably reticulate. For example, *S. incanum* will hybridize with *S. pubescens* (Rao & Rao, 1987), *S. violaceum* with *S. virginianum* (Daunay et al, 1991), and *S. macrocarpon* with *S. aethiopicum* (Omidiji, 1979). Moreover, *S. melongena* will hybridize with *S. incanum* (Lester & Hasan, 1991), *S. virginianum* (Rao, 1979), *S. violaceum* (Rao & Kumar, 1980), *S. macrocarpon* (Schaff et al, 1980) and *S. aethiopicum* (Omidiji, 1986). This further complicates the interfertility dynamics between brinjal and its relatives.

Untested species

Other spiny solanums in section *Melongena*: eg. *S. eleagnifolium*, *S. heteracanthum*, *S. rostratum*, (all found in India), section *Oliganthes*: eg. *S. cordatum* (India, Pakistan), *S. hovei* (India), and section *Torva*: eg. *S. barbisetum* (Bangladesh, India, Myanmar), *S. sarmentosum* (India, Indonesia and Malaysia) are in need of study and are likely to show some level of crossability with *S. melongena* (see Table 1).

The need for consistency in crossability studies

Interpretation of hybridization data has often been made difficult by erroneous identification of parent species, or nomenclature using outdated synonyms or rejected names. Furthermore, much of the information on crossing success is difficult to correlate. This is because of the heterogeneity of the various methodologies of the fertility assessments (Daunay et al, 1999). For example, some studies such as Rao (1979) and Rao & Kumar (1980) used production of seeded fruits as the criterion for crossing success. Other criteria were: seed germination to produce F1 plants (IIVR, 2007), pollen stainability of F1 progeny (Lester & Hasan, 1991; Pearce & Lester, 1979), pollen stainability of the F2 generation (Pearce, 1975), and production of F1 plants by embryo rescue (Daunay et al, 1999).

In many cases, a limited range of both *S. melongena* and wild relative accessions was tested, often examining crossability in one (sometimes unspecified) direction only. The conclusions that can be drawn from such studies are therefore limited. Any selective advantage brought about by hybridization will manifest in the F1 or subsequent generations, and study of the fitness and fertility of hybrid progeny is therefore crucial. Lester and Niakan (1986) defined successful crosses as those that naturally produce F1 plants of comparable pollen fertility to the progeny of selfings of the parents. On this basis, full interfertility between species would thereby be indicated by crosses that fulfil this criterion in both directions. Unfortunately, detailed information of this sort has rarely been provided in brinjal crossability studies. In addition, very few methodologies seem to account for the complications arising as a result of heterostyly in brinjal flowers. As seen above, this has important implications for pollination success.

In investigating crossability and introgression potential between transgenic crops and wild relatives, some up-to-date studies (eg. Mayerhofer et al, 2011) have examined a broad range of parameters which include: the production of viable and fertile F1 progeny via reciprocal crosses, the transference of transgenic DNA to F1 progeny and its mode of inheritance, and evidence of increased fitness (such as heterosis or increased weediness) of hybrids. Similarly detailed analyses have yet to be undertaken with *Bt* brinjal.

7. Potential consequences of release of *Bt* brinjal on plant biodiversity

There has been considerable interest in the potentially damaging effects on plant biodiversity of the release of *Bt* brinjal (eg. Andow, 2010; Chaudhary & Gaur, 2009; CSA, 2006; IIVR, 2007, 2008; Samuels 2011a, 2011b; Samuels & Shanmugam 2011; Singh, 2009; Sood et al, 2010; Tzotzos et al, 2009). Much of this has been related to issues whereby ecological balance could be upset by transfer of pest resistance transgenes from *Bt* brinjal to wild, weedy or cultivated relatives.

Increased weediness

In conferring pest resistance upon these relatives, these transgenes could induce a selective advantage, encouraging such plants to become overbearing weeds (Samuels, 2011a). Such aggressive growth, coupled with the strong tendency for spiny solanums to become adventives, could have detrimental effects on ecological balance and biodiversity. There has been one recorded laboratory attempt in India to assess the weediness of *Bt* brinjal: however this was simply measured by comparing the germination success (presumably of single accessions) of *Bt* brinjal with that of untransformed brinjal on two basic growth media, using 50 seeds for each accession (MOEF, 2011a). From these limited observations it was surmised that there are ‘no substantial differences between *Bt* and non-*Bt* brinjal for germination and vigour’ or ‘with regard to their weediness potential’ (MOEF 2011b).

A single field study was conducted to monitor the ‘aggressiveness and weediness’ of *Bt* brinjal compared with non-GE brinjal plants (MOEF, 2011a). One of the plots used for a gene flow study (Chaudhary & Gaur, 2009) was left undisturbed after harvesting the transformed brinjal crop, and irrigated and monitored for three months after harvesting. No germination of any *Bt* brinjal plants was observed during the study. (Unfortunately, the opportunity to examine weediness by growing on the original hybrid progeny was not taken up). From this, it was surmised that ‘*Bt* brinjal does not have any weediness/aggressiveness characteristics and behaves in a similar fashion [to] other conventional brinjal varieties’ (MOEF, 2011a). The original report seems to have been written in a ‘lackadaisical manner’ and parts of it are described as incomprehensible (CSA, 2006). The statement that ‘the Cry1Ac gene used in *Bt* brinjal event EE-1 confers no advantage to recipient plants in terms of aggressiveness or growth characteristics’ (GEAC, 2009) seems to relate largely to this investigation, and is therefore based on, at best, limited evidence.

Ferality

S. melongena itself is believed to form adventive populations that have diverged away from cultivated ones, and wild populations of brinjal are known to occur in India (Chaudhary, 1995; Sekara et al, 2007). It is not known whether *Bt* brinjal can become feral in a similar way. The taxon *S. insanum* (= *S. melongena* L. var. *insana* (L.) Prain) has been described as a feral form of *S. melongena* (Daunay et al, 2001a; Lester & Hasan, 1991). A broad range of variation in *S. insanum* seems to be evident in India (Karihaloo & Rai, 1995). This may be due to hybridization with cultivated *S. melongena* populations, or other wild species such as *S. cumingii*, with which it is fully interfertile. Furthermore, the distinction between *S. insanum* and *S. cumingii* is unclear (Karihaloo & Gottlieb, 1995) and the two taxa seem to intermerge morphologically. It is theoretically possible, then, for weedy forms of *Bt* brinjal, or successful crosses between *Bt* brinjal and non-*Bt* brinjal, to pass on the *Bt* transgene via introgression into feral forms or wild species of *S. melongena s.l.* Thus, a complex situation regarding interfertility, ferality and the potential for introgression of transgenes exists.

Selective advantage for brinjal relatives

The information in Table 1 shows that successful crosses between untransformed *S. melongena* and several sympatric wild relatives are possible. It has been argued that, even if the insect-resistance transgene is able to flow to wild relatives, it will not confer any fitness advantage, because insect pests such as the EFSB are ‘rarely found on them’ (GEAC, 2009). In contrast to this assertion, Waterhouse (1998) lists seven *Solanum* host species of the EFSB, two of which are capable of hybridizing with brinjal (*S. violaceum* and *S. virginianum*). In this case, transfer of pest resistance would confer a selective advantage, with potential consequences for increased weediness and consequent disruption of plant biodiversity.

Loss of agricultural and horticultural biodiversity

There is insufficient evidence that non-GE brinjal will remain uncontaminated, and this risk needs to be evaluated (Andow, 2010). The possibility of ‘impinging upon the right of farmers for safe and sustainable use of indigenous agro-biodiversity’ (Yadugiri, 2010) is a concern, whereby the genetic resources of many traditional cultivars and landraces could be compromised by transgene transfer. Certain traditional systems of medicine in India employ brinjal or some of its close relatives (Anand, 2006; Kameswara Rao, 2011) and there is further concern over genetic compromise of these much-valued treatments. Some would argue that brinjal itself is not a significant component in such preparations (Kameswara Rao, 2011), but nevertheless several close relatives, interfertile with brinjal, ostensibly are.

8. Conclusions and recommendations

Our knowledge of the diversity and taxonomy of the wild relatives of brinjal in South and South-East Asia is incomplete. There is thus a prime need to develop this knowledge, as a basic starting point in the understanding of the potential for transgene transfer involving GE brinjal.

Hybridization between *Bt* brinjal and some of its wild, weedy or cultivated relatives is likely if *Bt* brinjal is cultivated. However, evidence as to the precise interfertility relationships amongst these species, and between these species and brinjal, is in urgent need of updating so that a more detailed view of the potential for the introgression of *Bt* transgenes is gained.

Once such interfertility relationships are understood in more detail, we can then progress further in our evaluation of the risks to plant biodiversity. These relate to: (a) wild or weedy relatives of brinjal obtaining selective advantage via transgene transfer from *Bt* brinjal, (b) wild relatives of brinjal suffering reduced genetic diversity from the introgression of the *Bt* transgene, and (c) introgression of the *Bt* transgene into non-GE brinjal (Andow, 2010).

In many cases, data relating to the areas of concern in (a)-(c) above have been obtained from reports that have not been formally published. There has been much controversy over the reliability of such data, and this has been a major argument for withholding commercialization of the GM crop in India. Peer review (Yadugiri, 2010) of investigative studies via publication in internationally acknowledged scientific journals would enable all data to be considered on a credible and equal basis. Only after accumulation and consideration of unbiased scientific data can a healthy debate take place (Samuels & Shanmugam, 2011).

General concerns over transgene escape were incorporated into the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (SCBD, 2000) to which Bangladesh, India and the Philippines are signatories. Early in 2010, the Indian government incurred a moratorium on the commercialization of *Bt* brinjal in India, which continues. At the Tenth Conference of the Parties to the Convention on Biological Diversity (COP10) in Japan in October, 2010 a new ten-year Strategic Plan with 20 targets was constructed. Target 9 is geared towards preventing the introduction of invasive species, whilst Target 13 relates to conserving the genetic diversity of crops and their wild relatives (CBD, 2010). In accordance with COP10 guidance, and whilst the Indian moratorium continues, it is proposed here that more detailed and thorough consideration is given to the implications for plant biodiversity of the commercialization of *Bt* brinjal.

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