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Recent advances in naga king chilli (*capsicum chinense* JACQ.) research

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Abstract

The Naga King Chilli (*Capsicum chinense* Jacq) is one of the hottest chillies in the world. This chilli is native to the north eastern region of India and subsequently the geographical indication (GI) of goods tag for this chilli has been obtained by the Nagaland State Government. The chilli was recorded to be the hottest chilli in the world in 2006 with a Scoville heat unit (SHU) rating of 1,001,304. Currently it occupies the fifth position among the hottest chillies in the world. Due to its high potential commercial value, many studies has been carried out in this crop including scientific cultivation, *in vitro* regeneration, diversity and evolution studies and its diseases and their management etc. This review is an attempt to bring into account the various research work carried out so far in the crop including the traditional and ethno-medicinal uses.

Highlight

- Naga King Chilli is a result of gene introgression.
- *In-vitro* regeneration using different explants, diversity studies, scientific cultivation, disease studies etc is reported.

Keywords: Naga king chilli, GI, SHU, *in vitro* regeneration, evolution, diseases, ethno-medicinal

Chillies (*Capsicum* spp.) belong to the family Solanaceae (Nightshade family) and they are used variously as a pungent flavor in food, natural plant colour, pharmaceutical ingredient and as sprays for riot control and self-defence. There are about twenty two wild and five cultivated species of *Capsicum viz. C. annuum, C. baccatum, C. frutescens, C. chinense* and *C. pubescens* (Bosland 1994). It is believed that chilli originated from tropical South America (Greenleaf 1986), but are now grown worldwide. In India, *C. annuum* is most widely cultivated, whereas, the cultivation of *C. frutescens, C. chinense,* and *C. baccatum* is not common and are usually restricted to homestead gardening in different regions (Reddy *et al.* 2014).

The northeast region of India is recognized as hot-spot for chilli diversity (Mathur *et al*. 2000). Among the many landraces of chilli that are cultivated in the northeast, the Naga King Chilli is the best known worldwide. The Naga King Chilli is considered as India's hottest chilli and was previously acknowledged as the world's hottest chilli having Scoville heat units (SHU's) rating of 1,001,304 (Bosland and Baral 2007). A number of variants of this chilli are noted in the north-eastern region of India (Kumar *et al.* 2011) with different local names

such as Naga chilli in Nagaland, Bhut Jolokia in Assam, and U-Morok in Manipur (Sanatombi *et al.* 2010, Verma *et al.* 2013). This chilli is grown mainly in the state of Nagaland, Assam and Manipur and to some extent in Mizoram, Arunachal Pradesh and Meghalaya. The chilli is also cultivated in the north eastern region of Bangladesh (Bhuyan *et al*. 2015). Because of its commercial importance, the Nagaland Government obtained the Geographical Indication (GI) of Goods tag for Naga King Chilli in the year (Registration and Protection) Act 1999.

The Naga King Chilli mainly belongs to the species *Capsicum Chinense* Jaqc. However, it has been reported that the chilli is a naturally occurring hybrid and occupies a taxonomic position between *C. chinense* and *C. frutescens*, clustering more closely with *C. chinense* group (Boshland and Baral 2007). The plant is a self pollinated species, but considerable cross pollination (up to 10%) may occur in presence of high insect population and this behaves as a semi-perennial plant if grown under optimal condition (Borgohain and Devi 2007). The plant and fruit characters also show wide variability (Murmu *et al.* 2014).

Morphological Characteristics

- Plant Height 45 - 200 cm
- Stem colour: Dark Green
- Leaf : Green in color, ovate in shape
- Leaf length: 10 – 14 cm
- Leaf width: 5.5 – 7.5 cm
- Flower: pendant with creamy white corollas, often with a touch of light green.
- Annular constriction: Present below calyx
- Anther colour: Blue
- Filament colour: Purple
- Fruit colour at maturity: Red, brown, white red
- Fruit shape: Sub-conical to conical
- Fruit length: 5.5 – 8.5 cm
- Fruit width at shoulder: 2.5 – 3.0 cm
- Fruit weight: 4 – 10 g
- Fruit surface: Rough, wrinkle with spikes
- Number of locules in fruits: 4 – 5 with 25-35 slightly wrinkled seeds.
- Seed colour: Light brown
- 1000-seed weight: 0.43 – 48 g
- Seeds/ Fruit: 21 – 55 seeds, slightly wrinkled
- Hypocotyl colour: Green

Cultivation Practices

The Naga King Chilli is widely cultivated in north-eastern states of India predominantly in Nagaland, Assam and Manipur. Two planting seasons are practised *viz*. kharif and rabi. Kharif cultivation starts during February - March mainly in the hilly states whereas rabi crop is grown in the plains of Assam during September-October (Baruah *et al*. 2014).

In Nagaland, the farmers practise Jhum cultivation in paddy fields as a sporadic intercrop with summer paddy and also in small homestead garden. The crop is semi perennial but the fruits' size is gradually reduced beyond three years of growth. In homestead traditional gardens, the farmers prefer to grow the crop in the shade rather than in sunny places as it yields fruits with enhanced pungency. In Jhum cultivation, direct seeding is practiced in paddy fields during February-March and the peak harvest time is between August-September (Bhagowati and Changkija 2009).

The productivity and pungency of *rabi* grown crop is generally more than *kharif* grown crop (Borgohain and Devi 2007). Sowing of the crop during September produced more pungent fruits than sowing in the month of February (Sanju *et al*. 2012).

For sowing, the chilli pods are collected after maturation when they are bright red or orange in color, the seeds are then extracted and sun dried for at least three days. After drying, the seeds can be immediately germinated or stored in airtight containers/plastic bags. The germination of the seeds take about 15-20 days and therefore the seeds should be treated with fungicides and insecticides to avoid damage due to fungal or insect attack during the germination period. The crop can be grown under diverse soil condition but for optimum growth, it requires well drained sandy loam soil, clay loam or laterite soil (Borgohain and Devi 2007).

Ethno-Medicinal uses of Naga King Chilli

Bhagowati and Changkija (2009) reported some of the ethno-medicinal uses of Naga King Chilli as mentioned below:

- Used as relief for asthma patients at low quantities
- For treatment of gastro intestinal abnormalities by regular consumption in small quantities
- They are also used to tone up body muscles after heavy work exercise
- Hot infusions of Naga chilli are used for toothache and muscle pain
- Tender leaf pastes are applied as thin coat over boils for easy removal of pus from boils.

Commercial Prospect of the Crop

The secondary metabolite group, capsaicinoids, are produced solely in the fruit of members of the genus Capsicum (Stewart *et al.* 2005). Capsaicin and hydroxycapsaicin, the major constituents of capsaicinoids, are highly desirable and essential for spice, food, medicinal, and industrial purposes (Ochoa-alejo and Ramirez 2001). Capsaicin is also the active principle which accounts for the pharmaceutical properties of chillies and is useful as a counter-irritant, anti-arthritic, analgesic, anti-oxidant and anti-cancer agent.

Bhut Jolokia is characterized by very high capsaicinoid content, ranging from 2.45% (Sarwa *et al.* 2013) to 5.36% (Liu *et al*. 2010). As a result, it is an ideal chilli variety of India for extraction of capsaicin. Most other chilli varieties cultivated in India have less than 1% capsaicin and these are not suitable for capsaicin extraction since 1% capsaicin is a standard needed for its commercial extraction.

In Vitro Culture of Naga King Chilli

The *in vitro* regeneration of capsicum species is reported to be difficult (Liu *et al*. 1990, Ochoa-alejo and Ramirez 2001) but protocol needs to be developed and thus efforts are made especially for elite cultivars such as Naga King Chilli, for increased production as well as productivity. The Naga King Chilli being a recalcitrant plant, the *in vitro* culture response is very poor. Several workers have reported the *in vitro* regeneration of Naga King Chilli using different explants (Sanatombi and Sharma 2008 [a,b], Kehie *et al.* 2011, 2013) for direct regeneration and through callus (Raj *et al.* 2015, Gayathri *et al*. 2015) with various plant growth regulator (PGR) combinations and concentrations. Following are the works done so far for *in vitro* culture of Naga King Chilli.

Direct organogenesis from hypocotyls, cotyledons and whole leaf explants using Murashige and Skoog (MS) medium supplemented with a PGR combination of Benzyl amino purine (BAP) and indole-3-acetic acid (IAA) are reported. The most responsive medium for shoot induction is recommended to be the combination of 8.8 µM BAP and 11.4 µM IAA, with the cotyledon and leaf as explants being more responsive than hypocotyls. For shoot elongation and rooting, MS medium containing 2.8 µM BAPand 2.8 - 5.7 µM IAA or 2.5 - 4.9 µM Indole Butyric acid (IBA) is efficient (Sanatombi and Sharma 2008b).

Shoot tip and nodal segment explants have also been used for *in vitro* regeneration of Naga King Chilli. The use of MS media fortified with thidiazuron (TDZ) alone (18.16 µM) is efficient for shoot induction and proliferation for both the explants followed by BAP (35.52 µM). Preference of IAA (5.70 µM) rather than Naphthaleneacetic acid (NAA) for root induction is also reported (Kehie *et al*. 2011).

The induction of Rosette-like structures (RLS) on cotyledon explants using MS agar medium fortified with TDZ (18.16 µM), which later on were regenerated into complete plants is also reported, with the considerable amount of the capsaicin content (837,760 SHU), a little lower than that of normal *in vivo* raised plants (872,000 SHU) (Kehie *et al.* 2013).

Callus has also been induced from placental tissues of Naga King Chilli using MS medium supplemented with 2mg/L 2,4-D (2, 4 – dichlorophenoxyacetic acid) and 0.5mg/L Kinetin (Mangang 2014), producing a good amount of proliferation and friable callus. This finding is significant taking into consideration the fact that capsaicinoids are exclusively synthesized in chilli fruits, specifically in the placenta and the interlocular septum, where they accumulate in vesicles (Fujiwake *et al*. 1980, Stewart *et al*. 2007). Therefore, if plants are regenerated using placenta as explant, it may produce plants with enhanced capsaicinoid content.

Reports of regeneration of Naga King Chilli by indirect organogenesis are also available. Multiple shoot was initially induced using MS medium supplemented with 5 mg/L BAP and 0.5 mg/L IAA (Raj *et al.* 2015) or MS medium fortified with BAP

Table 1 Protocols developed for i*n vitro* culture of Naga King Chilli

Explants used	Type of culture	PGR combination/concentration used	Reference
Hypocotyls, cotyledon and whole leaf	Direct regeneration	BAP (8.8 µM) + IAA (11.4 µM) for shoot induction, 2.8 µM IAA for shoot elongation and IAA (2.8 - 5.7 µM) or IBA (2.5 - 4.9 µM) for rooting	Sanatombi and Sharma 2008b
Shoot tip and nodal segment	Direct regeneration	TDZ (18.16 µM) for shoot induction and proliferation and IAA (5.70 µM) for root induction	Kehie *et al.* 2011
Cotyledon	Formation of rosette like structures leading to shoot regeneration	TDZ (18.16 µM)	Kehie *et al.* 2013
Placental tissues	Callus induction	2mg/L 2,4-D and 0.5mg/L Kin	Mangang, R. J. 2014
Stem segments of *in vitro* raised plants	Regeneration *via* callus	3 mg/L BAP and 1 mg/L NAA for callus induction and MS basal medium for shoot elongation and rooting	Raj *et al.* 2015
Leaf of *in vitro* raised plants	Regeneration *via* callus	6.66 µM BAP with 9.05 µM 2,4-D for callus induction, MS basal medium for shoot elongation and 7.36 µM IBA for rooting	Gayathri *et al.* 2015
Inter nodal part of *in vitro* raised plants	Regeneration *via* callus	6.66 µM BAP with 8.06 µM NAA for callus induction, MS basal medium for shoot elongation and 7.36 µM IBA for rooting	Gayathri *et al.* 2015

*All workers listed above used Murashige and Skoog (MS) medium

**PGR = Plant Growth Regulators; BAP = Benzyl Amino Purine; IAA = Indole Acetic Acid; IBA = Indole Butyric Acid; TDZ = Thidiazuron; 2, 4-D = 2, 4-Dichlorophenoxyacetic acid; Kin = Kinetin; NAA = Naphthalene Acetic Acid.

Table 2: Diseases of Naga King Chilli

Disease type	Name of disease	Causal organism	Reference
Viral diseases	Mosaic disease	*Cucumber mosaic virus, Potato virus Y*	Talukdar *et al.* 2015, Baruah *et al.* 2016
	Leaf curl disease	*Chilli leaf curl virus*	Borgohain and Devi 2007, Talukdar *et al.* 2015, Baruah *et al.* 2016
	Bud necrosis disease	*Groundnut bud necrosis virus*	Baruah *et al.* 2016
	Veinal mottle disease	*Chilli veinal mottle virus*	Banerjee *et al.* 2013
	Leaf and stem necrosis disease	*Tomato spotted wilt virus*	Talukdar *et al.* 2015
Fungal diseases	Anthacnose / Fruit rot disease	*Colletotrichum capsici*	Borgohain and Devi 2007, Talukdar *et al.* 2015
	Die- back disease	*Colletotrichum gloeosporoides*	Talukdar *et al.* 2015, Borgohain and Devi 2007
	Stem rot and wilt disease	*Sclerotinia sclerotiorum*	Talukdar *et al.* 2015
	Collar rot disease	*Rhizoctonia solani*	Talukdar *et al.* 2015
	Leaf spot disease	*Corynespora cassicola*	Talukdar *et al.* 2015
	Damping-off disease of seedling; Root and stem rot in young transplants	*Rhizoctonia solani*	Ngullie and Daiho 2013
Bacterial disease	Bacterial wilt disease	*Ralstonia solanacearum*	Talukdar *et al.* 2015

alone or in combination with IAA and adenine sulphate (Gayathri *et al*. 2015). Callus were then induced from the *in vitro* raised plants using stem segments by culturing on MS medium fortified with 3 mg/L BAP and 1 mg/L NAA (Raj *et al.* 2015) and leaf or inter nodal part using MS medium containing 6.66 µM BAP with 9.05 µM 2.4.D and 6.66 µM BAP with 8.06 µM NAA, respectively (Gayathri *et al*. 2015). Shoot elongation and rooting were done in MS basal medium or MS medium fortified with 7.36 µM IBA.

Capsaicin production using suspension cultures of Naga King Chilli under osmotic stress conditions is possible. A combination of sucrose and NaCl led to increased accumulation of capsaicin as compared to sucrose alone. (Kehie *et al*.2012).

The above reports suggest that the nutritional requirement for different explants or plants collected from different locations may vary. The protocols developed for various explants (Table I) may now be used for micropropagation of the crop.

Genetics and Evolution of Naga King Chilli

When Naga King Chilli was first discovered, it was reported that it belonged to *Capsicum frutescens* sp. (Mathur *et al*. 2000). Later on, based on morphological characters using Capsicum descriptors developed by the International Plant Genetic Resources Institute (1995), Bosland and Baral (2007) confirmed that Naga King Chilli is a member of *C. chinense* species. They used Randomly Amplified Polymorphic DNA (RAPD) markers for species identification and concluded that genetic introgression occurred from *Capsicum frutescens* into Naga King Chilli and placed it in a taxonomic position between *C. chinense* and *C. frutescens*, clustering more closely with the *C. chinense* group.

The distinctiveness of 'Naga King Chilli' or 'Bhut Jolokia' from *Capsicum frutescens* or *Capsicum chinense* has been determined using ribosomal RNA gene- internal transcribed (ITS) region sequences. The phylogenetic analysis using ITS1, 5.8S and ITS2 sequences revealed the distinctness of Naga King Chilli from all other members within the genus and beyond. Moreover, a unique 13-base deletion in all the representative accessions of 'Bhut Jolokia' is also reported (Purkayastha *et al.* 2012). The ITS1-5.8S-ITS2 sequence also revealed the variability of ITS1 far exceeding that of ITS2 with respect to nucleotide diversity and sequence polymorphism and the 5.8S gene as a much conserved region. The Naga King Chilli population is evolving at drift–mutation equilibrium and are free from directed selection pressure suggesting an ancient population expansion of this chilli (Kehie *et al*. 2016).

The study of whole fruit proteome and differentially expressed proteins and/or gene products of Bhut Jolokia identified a total of 107 dominant proteins, of which 14 proteins showed distinct quality with unique expression in Bhut Jolokia and 6 proteins exhibiting quantitative differential expression alterations (Purkayastha *et al.* 2014).

Diseases of Naga King Chilli

Naga King Chilli or Bhut Jolokia is vulnerable to several biotic stresses caused by virus, fungus and bacteria (Talukdar *et al.* 2012). During the crop growth several diseases infest the plants, the most common diseases being 'die-back', 'anthracnose' and 'leaf curl' (Borgohain and Devi 2007). A list of types of diseases infecting Naga King Chilli and their causal organism is given in Table II.

In Assam, the incidence of viral infection in Bhut jalokia is highest (60%) as compared to fungal infection (10%) and bacterial infection (3%) (Talukdar *et al.* 2015). The Naga King Chilli is susceptible to different viruses such as *Cucumber mosaic virus* (CMV), *Potato virus Y* (PVY) (Talukdar *et al.* 2015, Baruah *et al.* 2016), *Tomato Spotted Wilt Virus* (TSWV) (Talukdar *et al.* 2015), *Chilli leaf curl virus* (ChLCV) (Borgohain and Devi 2007, Talukdar *et al.* 2015, Baruah *et al.* 2016) and Groundnut Bud Necrosis Virus (GBNV) (Baruah *et al.* 2016). The susceptibility of Naga Chilli to *Chilli veinal mottle virus* in Meghalaya is also noticed (Banerjee *et al.* 2013). The plants may be infected by single virus or more than one virus together forming a disease complex (Baruah *et al.* 2016).

Many fungal diseases also affect Naga King chilli such as Anthracnose/Fruit rot caused by *Colletotrichum capsici* and Die-back caused by *Colletotrichum gloeosporoides* (Borgohain and Devi 2007, Talukdar *et al.* 2015), Stem rot and Wilt caused by *Sclerotinia sclerotiorum*, Collar Rot caused by *Rhizoctonia solani* and Leaf spot caused by *Corynespora cassicola* (Talukdar *et al.* 2015).

Among the bacterial infection, the bacterial wilt disease caused by *Ralstonia solanacearum* is observed at later stages of crop growth (Talukdar *et al.* 2015). Contradictory to the above mentioned susceptibility of Naga King Chilli to various patyhogens, some reports are available for the resistance/tolerance of Bhut Jolokia to certain pathogens such as *Pepper leaf curl virus*-Varanasi strain (Kumar *et al.* 2006, 2011) and *Colletotrichum* spp. (Garg *et al.* 2012).

Disease Management Studies

Viral diseases of Naga King Chilli can be managed by an integrated approach including treatments in nursery and main field condition. The seed treatment with imidacloprid@0.25 ml/l + nursery net+ foliar spray with imidacloprid @ 2ml/l at 15, 30, 45, 60 DAT is most efficient to combat the infection of the plants due to viral disease complex by actually reducing disease incidence and vector population count (Baruah *et al.* 2016).

The fruit rot disease in Naga King Chilli caused by *Colletotrichum gloeosporioides* can be controlled by using a combination of *Trichoderma viride* and *Pseudomonas fluorescens*, the biocontrol agents, which inhibit mycelial growth of the fungi and reduce prevalence of disease (Ngullie *et al*. 2010). The same combination of *T. viride* and *P. Fluorescens* is also efficient in reducing the incidence of seedling rot caused by Rhizoctonia solani (Ngullie and Daiho 2013). Plant extracts of *Allium sativum* (10%) and *Azadirachta indica* (10%) also inhibit mycelial growth of *C. gloeosporioides*. However, the best result is obtained by use of Bavistin (0.1%) with 80.84% disease reduction (Ngullie *et al*. 2010).

The efficacy of biocontrol agents in controlling bacterial diseases of Naga King Chilli has been explored. A combination of *T. viride* + *P. fluorescens* is also effective in suppressing bacterial wilt incidence and promoting plant growth of Naga King Chilli (Kataky *et al.* 2016).

Conclusion

There is no doubt that the Naga King Chilli, being one of the hottest chilli in the world, has enormous potential for commercialization. Even though commercialization of this particular crop has been started, it is still at a nascent stage. The importance of this crop is magnified not only because of its high capsaicin content but also due to its medicinal use. Significant progress has been made in Naga King Chilli research such as scientific cultivation of the crop, development of *in vitro* regeneration protocols using different explants, identification of important diseases of the crop and its mitigation etc, but still there is a long way to go. The finding that Naga King Chilli is a result of gene introgression from *C. frutescens* into *C. chinense* favour the prospect of doing wide hybridisation among the Capsicum species for development of elite genotypes. Use of biotechnological methods to produce biotic or abiotic stress tolerant Naga King Chilli is another area of research which needs to be explored to enhance the productivity of the crop.

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