VEGETATIVE PROPAGATION OF Azadirachta indica A. JUSS (NEEM) THROUGH CUTTINGS: A REVIEW

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ABSTRACT: Azadirachta indica A. Juss (Neem) is one of the important multipurpose tree species. Today, neem is receiving worldwide recognition for its variety of bioactive principle components. Neem, being an important part of our biological heritage and is also recognised as one of nature’s gift to mankind. This review aims to perform a literature review of the main scientific methods used to obtain clones of Azadirachta indica, such as the processes of vegetative (macro) propagation and present perspectives and future trends for the application of new cloning techniques aiming for large scale for plant production. The literature describes methods (dip treatment), types of plant growth regulator (IBA, NAA & IAA), types of stem cuttings (hard wood, semi hard wood, soft wood, leafy & mini cuttings) and planted in rooting media (sand, soil, vermiculite & sand+soil+FYM) during different season (monsoon, winter & summer). The improvement aiming the disseminating such techniques can minimize costs, shorten production stages and consequently, reduce the cultivation time in the laboratory.

Keywords: Azadirachta indica, vegetative propagation, cuttings, auxin.

PROPAGAÇÃO VEGETATIVA DO NIM INDIANO (Azadirachta indica A. Juss) POR MEIO DE ESTacas: UMA REVISÃO

RESUMO: Azadirachta indica A. Juss (nim indiano) é uma importante espécie florestal que apresenta múltiplos usos. Hoje, o nim está recebendo o reconhecimento mundial por sua grande variedade de compostos bioativos. Considera uma herança biológica significativa, também é reconhecida como um dos presentes da natureza para a humanidade. Este trabalho tem como objetivo realizar uma revisão da literatura sobre os principais métodos científicos utilizados para se obter clones de Azadirachta indica por meio de propagação vegetativa, avaliado perspectivas atuais e as tendências futuras para a aplicação de novas técnicas de clonagem para a produção em grande escala. A literatura descreve métodos de tratamento (mergulhia), tipos de regulador de crescimento (IBA, NAA e IAA), tipos de estacas (madeira dura, madeira semi dura, madeira macia, folhas e mini estacas) e enraizamento (areia, solo, vermiculita e areia + solo + FYM) durante diferentes épocas (estação chuvosa, inverno e verão). A divulgação de tais técnicas podem minimizar os custos, encurtar as etapas de produção e, consequentemente, reduzir o tempo de cultivo em laboratório.

Palavras-chave: Azadirachta indica, propagação vegetativa, estacas, auxina.

1. INTRODUCTION

Azadirachta indica A. Juss (Neem), belongs to family Meliaceae, is widely grown in South Asia, Southeast Asia and West Africa. Azadirachta indica is an evergreen tree of 10 -15 m in height, with pinnate leaves up to 3-10 cm long. Flowering occurs during April and May is related to high temperatures and low rainfall. The floral parts rise acropetally. Inflorescence is long, slender, axillary or terminal panicules with abundant white, or pale-yellow, fragrant flowers that are 0.3 to 0.4 cm across. Fruits are smooth, green, ellipsoidal drupes, 1.2 to 1.8 cm long, and 1.0 cm wide that turn yellow to brown when ripe. The seeds ripen from June to August. A single tree of 10 to 12 years may produce 5 to 8 kg of seeds annually, while fully grown trees 20 years or older produce 30-35 kg of seeds. Azadirachta indica seeds are ovoid or spherically pointed apically with a thin testa. The seed is exarillate with a small adaxial sacrotesta (PENNINGTON; STYLES, 1975). Neem acts on insects in several ways including contact toxicity, repelling adults and larvae, disrupting developmental processes, and disrupting adult behaviour such as mating (ISMAN, 2006). Products of the neem tree (Azadirachta indica A. Juss) from all parts of the plant have demonstrated efficacy against many pest species.
including arthropod pests and diseases of crops. Neem also acts systemically because of absorption by roots and translocation to plant parts when applied into soil or sprayed on the plant (THOEMING et al., 2006). The principal active ingredient, Azadirachtin, is however more concentrated in the kernel, which makes it the most effective part of the plant (SCHMUTTERER, 1990; GAHUKAR, 2000). A broad variety of high free fatty acid content of vegetable oils is available in Azadirachta indica, which conveys great potential sources for biodiesel production in India (DEEPAK et al., 2013).

2. REVIEW

2.1. Vegetative Propagation

Vegetative propagation by cuttings is widely used to multiply elite trees obtained from the natural population to exploit the genetic variability (HARTMANN et al., 2011). The development of vegetative propagation techniques reduce the variability and at the same time ensure increased productivity. For the production of high quality timber and faster tree growth, it is essential to start vegetative propagation programme by selecting elite clones or genotypes from which the shoot cuttings are to be taken. Vegetative propagation through stem cuttings is the most vital method to reproduce plants and conserve their innate desirable characters. Establishment and growth rate of the cutting depends on seasonal and age variation, portion and diameter of stem, growing media, moisture level, nutrient status and temperature.

In the vegetative propagation, propagators usually select healthy, vigorous, well-matured and young shoots with viable buds as the source of cuttings. The degree of maturity and juvenility of stem cuttings plays an important role in rooting. Several types of stem cuttings can be taken from the parent donors, which may be of hard, moderately hard, soft or herbaceous, depending on maturity of branch (HARTMANN et al., 2011). In addition, the use of plant growth regulators plays an essential role in influencing the formation of adventitious root, bud break and survival of stem cuttings. Adventitious rooting in cuttings has long been known to be affected directly by auxins, which can be either naturally occurring within the plant (endogenous) or applied to the plant (exogenous) during vegetative propagation. Usually IBA and NAA are recommended to promote adventitious roots in cuttings from shrubs or trees (HUSEN; PAL, 2003).

Adventitious root formation in cuttings has many practical implications in forestry and there is a lot of commercial interest from the tree improvement point of views (PAL, 1995). Formation Adventitious root involves the process of redifferentiation, in which predetermined cells switch from their morphogenetic path to act as mother cells for the root primordia initiation (AESCHBACHER et al., 1994). Once root primordia have been initiated in cuttings, then considerable metabolic activity occurs as new root tissues appeared and the roots grow through and out of the surrounding stem tissues. Among these activities, the process of lignification in the cell wall, catalyzed by a particular peroxidise, occurs during the rooting (MCDOUGALL, 1992; SATO et al., 1993). Auxins have been shown to regulate different aspects of plant growth and development by affecting numerous processes, including cell division, cell elongation and differentiation (SRIVASTAVA, 2002). Applications of auxins to shoot cuttings play an important role in metabolic changes during the initiation, emergence and root primordia development. Auxin plays an important role in mobilization of carbohydrates in leaves and upper stem, also increase transport to the rooting zone (HAISSIG, 1986). Auxins will increase the availability of sugar at the site of root formation (ALTMAN, 1972) by increasing mobilization of starch through increased activity of hydrolyzing enzymes. The current rate of photosynthesis may also contribute and translocate sugar to the base of cuttings and thus play an important role in adventitious root formation of certain species (DAVIS; POTTER, 1981). The acetic acid (IAA) promoted the rooting of cuttings because it increases sugar availability at the site of primordium development. An increase in the activity of hydrolyzing enzyme following auxin treatment has been reported by many workers (HAISSIG, 1986). Loss of carbohydrates from the rooting zone of cuttings indicates that sugars are utilized during the root growth (HUSEN; PAL, 2003). Enzymes, which are known as metabolic markers exhibits change during plant development and differentiation (BHATTACHARYA, 1988). The activities of enzymes in the rooting zone of cuttings may provide an easy, fast and reliable means of assessing cellular differentiation in the roots. High humidity environments created by means of mist systems are commonly used in vegetative propagation experiments to reduce the risk of water stress (HARTMANN et al., 2011; NEWTON; JONES, 1993; JINKS, 1995). High humidity has been shown to enhance rooting capacity. The higher temperature of the environment suggests that the increase in root and leaf biomass could reflect enhanced photosynthesis (AMINAH et al., 1997). Rooting capacity of different cutting types depended on collection season. Seasonal variation in rooting capacity is very common in plants (HARTMANN et al., 2011; SWAMY et al., 2002). This may be because the accumulation of reserve material (carbohydrates) and growth regulators is different in different seasons (HARTMANN et al., 2011).

The high root formation could be attributed probably due to appropriate water holding capacity and good aeration of the rooting media. Aeration also plays a very significant role in numbers of root initiation and as well as on root elongation (KHAYyat; SALEHI, 2007). EL- NAGGAR; EL-NASHARTY (2009) reported that potting media as well as nutritional requirements are the most important factors affecting growth of ornamental plants. KHAYyat; SALEHI (2007) observed that the type of rooting media and their characteristics are of utmost importance for the quality of rooted cuttings.

2.2. Vegetative (Macro) propagation of Azadirachta indica A. Juss (Neem)

In neem, raising plants from seed is an easy and conventional method of propagation. The neem seed does not store well and its viability falls rapidly after two weeks. There is wide variability in the planting stock raised from seed. Vegetative propagation helps in early setting of uniform plantable nursery stock in comparison to sexual reproduction and results in rapid multiplication.

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Vegetative propagation of Neem allows for the mass production of genetically identical individuals (clones). Traditional methods of vegetatively propagated neem include grafting, root cuttings, stem cuttings and stump cuttings. The importance of vegetative propagation has increased during the last two decades as more attention is being paid to forest tree improvement programmes. Vegetative propagation technology helps a great deal in producing true to type plants for better quality and higher yield. As the traditional tree breeding methods will take very long time to deliver results, the easiest way to get immediate yield improvement is to raise clonal propagation of a few ‘superior types’ carefully selected from the wild population of neem. The efforts for development of suitable technology of vegetative propagation for rapid and mass production of clonal materials are at its beginning. Detail of research work on vegetative propagation of Azadirachta indica are described below:

Sivagnanam et al. (1989) reported that stem cuttings of neem dipped into IAA and IBA rooted effectively in mist propagator in 135 days. Leaf less hard wood cuttings of mature neem (15-20 cm long and 1-2 cm in diameter) collected during summer months, treated with 100ppm IBA for 24 hour took about 10-12 weeks to root but percent rooting was very low. Pal et al. (1992) has used clonal propagation aspects and designate that it a hard to root species. He studied on stimulation of adventitious root regeneration on semi hard wood, leafy soft wood cuttings of neem using auxins IBA, NAA, carboxylic acid and salicylic acid. Auxin treated and untreated (control) semi hard wood cuttings failed to root, however 30% of leafy softwood cuttings rooted even without treatment.

Kijar (1992) studied rooting ability of coppice shoots of Azadirachta indica and found rooting success of 90% for 100ppm IBA treated cuttings and 62% for untreated cuttings under mist. Reddy et al. (1993) reported that rooting response of shoot cuttings of Azadirachta indica in different rooting media showed that ordinary sand was best medium followed by vermiculite and 1000ppm IAA produced best rooting. Pal et al. (1995) studied on cheap Non-Auxin chemicals for rooting hard wood cuttings of Neem. They study the effect of IBA and Non-Auxinic chemicals (Potassium Permanganate, cobalt chloride and ascorbic acid) on the rooting. The results revealed that rooting was found in hard wood cuttings (1-2 cm in diameter and 15-20 cm long), when cutting treated with 100 mg/l IBA solution for 24 hour by basal dip methods and planted under high humidity conditions with shade exhibited rooting after 10-12 weeks. Kamaluddin; Ali (1996) investigated the effect of both leaf area in auxin on the rooting and growth of green stem cuttings from 2-year old Azadirachta indica tree. They concluded that treatment with 0.2% and 0.4% IBA solution has a significant effect on root development and the subsequent growth of the cuttings.

Palanisamy et al. (1996) studied on seasonal effect on induction of adventitious rooting in stem cutting of neem (Azadirachta indica). The stem cuttings of Azadirachta indica prepared during August-December (vegetative stage), February (bud breaking stage), march (flowering stage) and May-June (fruiting stage) were treated with water (control), 1000, 2000, 3000 ppm of IAA, IBA, thiamine and boric acid separately. Leaf fall or bud breaking stage was reported as best season and 1000 ppm IBA quick dip most suitable treatment for root induction in stem cuttings. VERMA et al., (1996) reported that hard wood stem cuttings (20-25 cm long and 1.0-1.5 cm in diameter) of Azadirachta indica, which prepared during spring season were treated with IBA and IAA (100, 500 and 1000 ppm) and distilled water. They observed that IBA treated cuttings were more effective than NAA treated once in producing roots.

Palanisamy; Kumar (1997) reported the influence of endogenous auxin on rooting of stem cuttings of Azadirachta indica. They concluded that rooting was dependent on endogenous auxin levels of the shoot and that longer cuttings (25cm long) rooted more readily than shorter cuttings (5cm). Gera et al. (1997) reported vegetative propagation of Azadirachta indica. Branch cuttings (22 cm long and 1.5 cm in diameter) treated with water (control), KMnO4 (1000 ppm), ascorbic acid (5000 ppm), KMnO4 (1000 ppm) + IBA (1000 ppm), ascorbic acid (5000 ppm) + IBA (1000 ppm) and IBA (1000 ppm) for 30 second. All treatments enhanced rooting over the control but IBA treated cuttings gave maximum (70%) rooting.

Tomar (1998) found stem cuttings rooted effectively with IBA but coppice shoots gave better result than cuttings from the main woody stems of Azadirachta indica. Palanisamy et al. (1998) reported that maximum rhizogenesis in stem cuttings coincided with the emergence of new shoots. Shoot cuttings of Azadirachta indica treated with IAA, IBA, B-vitamins and thiamine pyridoxine (1000, 2000 and 3000 ppm each) for 30 second and IAA, IBA and NAA (200, 500 and 800 ppm) for 15 hours. 85% rooting was observed with 1000 ppm IBA during February and other treatments produced comparatively less rooting in the branch cuttings.

Parthiban et al. (1999) reported that Azadirachta indica stem cuttings with double node segment gave effective rooting with IBA (2000 ppm and 3000 ppm) under 70% - 80% relative humidity. Singh; Chander (2001) reported that hard wood, semi hard wood and soft wood (22.5 cm long and 0.8 cm to 1.25 cm diameter) of neem with 0, 500, 1000 and 1000 ppm of IBA, IAA, 2, 4-D and their combination solution for 5 minutes. They observed maximum rooting in soft wood and semi hard wood cuttings treated with 500 ppm of IBA.

Palanisamy; Kumar (2001) reported that branch cuttings (25 cm long ,1.0 -1.5 cm of diameter) of neem were taken from 20 year old and treated with water (control), 1000, 2000 and 3000 ppm IBA, IAA and thiamine solution for 30 second. 1000ppm IBA induced 80% rooting and luxuriant root system during leaf fall season. Devarnavadagi et al. (2005) studied on effect of growth regulator on induction of adventitious rooting in stem cutting of neem. 1000 ppm IBA treatment for 10 minutes recorded higher sprouting (77.67%), rooting (51.33%) after 15 days. Ehiagbonare (2007) reported vegetative propagation studies on some key medicinal plants for malaria treatment in Nigeria were carried out. Vegetative propagation using 50 ppm indole-3-butyric
acid (IBA) showed significant difference in response to the effect of IBA in rooting stem cuttings. IBA (50 ppm) showed 70% rooting. Gehlot et al., (2014) reported vegetative propagation of Azadirachta indica through mini cuttings. The results showed that 250 mg L⁻¹ IBA treatment showed better results with sand in terms of 80% rooting percent, 70.63 number of roots, 11.13 root length and 5.25 number of leaves.

Several worker have reported regeneration of Azadirachta indica using stem cuttings are Sivagnanam et al. (1989), Kijkar (1992), Pal et al. (1992), Pal et al. (1994), Sharma et al. (1995), Palanisamy; Kumar (1996), Kamaludin; Ali (1996), Shanthi et al. (1996), Gera et al. (1997), Palanisamy; Kumar (1997), Gera et al. (1998), Palanisamy et al. (1998), Puri; Swamy (1999), Parthiban et al. (1999), Singh; Chander, (2001), Palanisamy; Kumar (2001), Palanisamy et al. (2003), Bhola (2004), Devarnavadagi et al. (2005), Gil et al. (2006), Ehiagbonare (2007), Reddy et al. (2007) and Gehlot et al. (2014). The details of mode of propagation, types of cuttings, rooting media, plant growth regulator used, methods & time, season, observation and results are described in Table 1.

3. CONCLUSION

Azadirachta indica is an important plant species and is in demand for planting in every climatic condition for multipurpose uses. In the plant propagation through macro cloning there is a need to maximize the output in term of higher successive rate and to minimize the input for multiplication at large scale to achieve the desired goal. In the present case, vegetative propagation through hard wood, semi hard wood and mini-cuttings was found to be a very promising method for large scale propagation.

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Table 1: Details of research work on vegetative propagation of *Azadirachta indica* A. Juss (Neem).

| S.No. | Authors and Year | Mode of Propagation | Type of Cuttings | Rooting Media | PGR used | Methods & Time | Season | Observation & Results |
|-------|------------------|---------------------|------------------|---------------|----------|----------------|--------|------------------------|
| 1     | Sivagnanam et al, 1989 | Stem Cuttings | Hard wood cuttings | - | IBA treatment for 24 hour | - | summer months | 72% rooting observed in hard wood cutting with treatment of 1000ppm IAA |
| 2     | Kijkar, 1992 | Stem Cuttings | Coppice shoots | - | IBA | - | - | 92% rooting observed in coppice shoots with treatment of IBA 100ppm |
| 3     | Pal et al, 1992 | Stem Cuttings | Leafy soft - wood cutting & Semi - Hard wood | Sand+Soil | IBA, NAA, IBA+NAA, Carbolic acid and salicylic acid | Quick Dip method & 24 Hour treatment | July (Monsoon) | No rooting in semi - hard wood cuttings. 34% rooting was observed in leafy soft - wood cutting with treatment of Carbolic acid (100 mg/l) |
| 4     | Pal et al, 1994 | Stem Cuttings | Terminal and sub terminal Leafy soft wood cuttings | Vermiculite | IBA (1000ppm), Potassium permanganate (1000ppm), Cobalt chloride (1000ppm) Ascorbic Acid (5000ppm) and their combinations | Basel dip method for 30 min treatment | June (Monsoon) | 50% Rooting was observed with treatment of Potassium permanganate + IBA (1000ppm) |
| 5     | Palanisamy and Kumar, 1996 | Stem Cuttings | Semi - Hard wood | Sand | IAA, IBA, B vitamin (thiamine) and Boric acid with concentration 1000,2000 & 3000ppm | Dip method | August, September, October & December (1993), February & March and May & June (1994). Effect of season were studied | 80% rooting was observed when semi hard wood cuttings treated with 1000 ppm IBA during February and best 100% sprouting achieved in treatment of 1000ppm boric acid |
| 6     | Kamaluddin and Ali, 1996 | Stem Cuttings | Green Stem Cuttings with 30, 50 & 100% leaf area | Sand | IBA with 0.2%, 0.4% & 0.8% concentration | Dip method for 2 - 3 sec | May | Cuttings treated with 0.4% IBA and 100% leaf area showed best Shoot length, stem dry weight, root number and root dry weight |
| 7     | Shanthi et al, 1996 | Seedling explant | Seedling explant | Sand | No PGR used | - | - | After 15 days of experiment rooting were observed in seedling explant |
| 8     | Gera et al, 1997 | Stem Cuttings | Branch cuttings | Sand | Kmno4 (1000ppm), Ascorbic acid (5000ppm), Kmno4 (1000ppm)+IBA (1000ppm), Ascorbic acid (5000ppm)+IBA (1000ppm), IBA (1000ppm) | Quick Dip method for 30 sec treatment | - | 70% Rooting observed in branch cuttings when treated with 1000ppm IBA |
| 9     | Palanisamy and Kumar, 1997 | Stem Cuttings | Stem cutting divided in three ends i.e. Distal, Middle and Proximal end with three diameter 0.5, 1.0, 2.0cm and three length 5, 12, 25cm | Sand | IBA (1000ppm) | Basel dip method &2 sec treatment | February | 100% rooting was obtained in cutting ( Distal end with 0.5cm diameter and 25cm length) with treatment of 1000ppm IBA |
| 10    | Gera et al, 1998 | Stem Cuttings | Juvenile shoot cutting collected from 10 indigenous provenances | Sand | IAA, IBA and B vitamin (thiamine) with concentration 1000ppm | Quick Dip method for 30 sec treatment | March | Cutting collected from 10 provenance showed an average 81% rooting, with treatment of IBA 1000ppm |
| 11    | Palanisamy et al, 1998 | Stem Cuttings | Semi - Hard wood 1.0 to 1.5 cm diameter & 25 cm length | Sand | IAA, IBA and B - vitamin, thiamine and Pyridoxine with concentration 1000ppm,2000ppm | Quick Dip method for 30 sec treatment | June/July 1993, April/May 1993 | 80% rooting was obtained in cutting collected during February and treated with 1000ppm IBA |
|   | Study                  | Material      | Cutting Type                              | Concentration | Treatment Method | Season         | Rooting Rate (Treatment)                             |
|---|------------------------|---------------|-------------------------------------------|---------------|------------------|----------------|-----------------------------------------------------|
| 12| Puri and Swamy, 1999   | Stem Cuttings | Branch cuttings 1.0 to 1.5 cm length      | Sand and Soil | Dip method       | July (Monsoon) | 68.7% Rooting obtained with treatment of 500 mg/l IBA |
| 13| Parthiban et al, 1999  | Stem Cuttings | Branch cuttings                          | Sand+Soil+FYM| Quick Dip method |   -             | 20 % Rooting obtained with treatment of 2000ppm & 4000 ppm IBA |
| 14| Singh and Chander, 2001 | Stem Cuttings | Hard wood, Semi-Hard wood & Soft wood    | IAA, IBA, 2.4-D and their combination with concentration of 500ppm, 1000ppm & 2000ppm | Dip method | July (Monsoon) | 33.3% Rooting was observed in soft wood cutting when treated with 500ppm IBA |
| 15| Palanisamy and Kumar, 2001 | Stem Cuttings | Branch cutting 1.5 to 1.5 cm diameter & 25 cm length | Not mentioned | Quick Dip method for 30 sec treatment | Different season (February, May, June, July, September & December) | Cutting collected in February showed 80% rooting with treatment of 1000ppm IBA |
| 16| Palanisamy et al, 2003 | Stem Cuttings | Semi-Hard wood cutting with 1.5 to 1.5 cm diameter & 25 cm length | Sand | Dip method | February | Cutting collected in February showed 100% rooting with treatment of 1000ppm IBA |
| 17| Bhola, 2004            | Stem Cuttings | Branch cuttings collected from selected five CPT's from 3 provenance | Sand+Soil+FYM(1:1:1) | Quick Dip method for 1 min treatment | August | Cutting collected from Bolangir provenance showed 58.40% rooting in cutting with treatment of 1000ppm IBA |
| 18| Devarnava dagi et al, 2005 | Stem Cuttings | Terminal, mid and epicormic shoot cuttings | IBA (500, 1000 and 1500ppm) | Quick Dip method for 5, 10 and 15 min treatment | July to October 1998 and 1999 | Cutting collected in February showed 80% rooting with treatment of 1000ppm IBA |
| 19| Gill et al, 2006       | Stem Cuttings | Hard wood cutting with 3-4 nodes and 0.5 - 1.0 cm thick | IBA (100, 200, 300 & 400ppm) | Quick dip method using IBA and IAA (1000, 2000, 3000 & 4000ppm) for 10 second and long dip methods using IBA and IAA (100, 200, 300 & 400ppm) for 12 hour period | July and February 2001 | 63.75% Rooting was observed in epicormic cutting when treated with 1000ppm IBA |
| 20| Ehiagbonar e, 2007    | Stem Cuttings | Single nod cuttings                      | Soil for IBA treated cuttings and sand for control | IBA (50 ppm) | Dip method for 10 second | - | 63.3% rooting was achieved after 12 weeks of experiment |
| 21| Reddy et al, 2007     | Stem Cuttings | Mid, Hard wood and Epicormic cuttings of 10-12 cm long with 2-3 nodes | Vermiculite | - | summer (April to May) and winter (November to December) | Epicormic cuttings collected during summer season showed 95% rooting with treatment of 1000ppm IBA |
| 22| Gehlot et al, 2014    | Stem Cuttings | Mini-cuttins                            | Sand, soil and vermiculite | IBA, IAA and NAA (100, 250, 500, 750, 1000 & 1500 ppm) | Dip method for 10 min | Mini cuttings collected during summer season showed 80% rooting with treatment of 250 ppm IBA |

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