



Emerging roles of NAC transcription factor in medicinal plants: progress and prospects

Ramesh Kumar^{1,2} · Shantanu Das¹ · Madhvi Mishra¹ · Debjani Roy Choudhury¹ · Komal Sharma³ · Abha Kumari² · Rakesh Singh¹

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Abstract

Transcriptional factors act as mediators in regulating stress response in plants from signal perception to processing the directed gene expression. WRKY, MYB, AP2/ERF, etc. are some of the major families of transcription factors known to mediate stress mechanisms in plants by regulating the production of secondary metabolites. NAC domain-containing proteins are among these large transcription factors families in plants. These proteins play impulsive roles in plant growth, development, and various abiotic as well as biotic stresses. They are involved in regulating the different signaling pathways of plant hormones that direct a plant's immunity against pathogens, thereby affecting their immune responses. However, their role in stress regulation or defence mechanism in plants through the secondary metabolite biosynthesis pathway is studied for very few cases. Emerging concern over the requirement of medicinal plants for the production of biocompatible drugs and antibiotics, the study of these vast, affecting proteins should be focused to improve their qualitative and quantitative production further. In medicinal plants, phytochemicals and secondary metabolites are the major biochemicals that impose antimicrobial and other medicinal properties in these plants. This review compiles the NAC transcription factors reported in selected medicinal plants and their possible roles in different mechanisms. Further, the comprehensive understanding of the molecular mechanism, genetic engineering, and regulation responses of NAC TFs in medicinal plants, can lead to improvement in stress response, immunity, and production of usable secondary metabolites.

Keywords *Andrographis paniculata* · Gene expression · Secondary metabolite · Signal transduction · Transcriptional regulation

✉ Rakesh Singh
rakesh.singh2@icar.gov.in
<http://www.nbpg.ernet.in/Personnel/Scientists/sid/87.aspx>

Ramesh Kumar
ramesh.manglesha@gmail.com

Shantanu Das
shanubdr.oryza@gmail.com

Madhvi Mishra
mahim190@gmail.com

Debjani Roy Choudhury
roydebj@gmail.com

Komal Sharma
komalgaur03@gmail.com

Abha Kumari
akumari@amity.edu
<https://www.amity.edu/aib/Faculty/Resumes/Abha-Kumari.pdf>

¹ Division of Genomic Resources, ICAR-National Bureau of Plant Genetic Resources, New Delhi 110012, India

² Amity Institute of Biotechnology, Amity University Uttar Pradesh, Uttar Pradesh, Noida 201313, India

³ Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi 110021, India

Introduction

Plants are the supreme producers of several small natural compounds called secondary metabolites, which are extensively utilized in the food, industry and pharmaceutical business. Secondary metabolites are known to be involved in plant defence mechanisms, interaction with other organisms and many medicinal aspects (Dixon 2001; Goossens et al. 2003). They also provide tolerance to the plant against some abiotic stresses, such as salinity, drought, UV irradiation, oxidative (ROS) and high light stresses (Ramakrishna and Ravishankar 2011). For example, flavonoids, terpenoids and alkaloids are some of the secondary metabolite's classes which serve several health benefits to humans as food ingredients or pharmacological agents (Goossens et al. 2003; Kennedy and Wightman 2011).

Medicinal plants contain secondary metabolites with antimicrobial and medicinal properties, due to which they are extensively studied to improvise them to treat several human diseases. The concept of biocompatible drugs, biopharmaceuticals and herbal formulations will enable the elimination of damages caused by multidrug resistance and other side effects of the available therapeutics. Table 1 represents some useful drugs derived from known medicinal plants (Bhutani and Gohil 2010; Mans et al. 2000). These natural products derived from medicinal plants exhibit anti-diabetic, anti-inflammatory, antimalarial, cardiovascular and antimicrobial (bacterial, fungal and viral) properties. Various biosynthetic and signalling pathways are involved in producing these metabolites containing different hormones, enzymes and feedback mechanisms. Several transcription factors regulate these pathways. The coordinated control of secondary metabolite production via transcription factors is the emerging field to analyze

the net production, evoking signals and controlling the signalling step. The molecular analysis of the transcription factor-based regulation would explore the methods to upscale the production of valuable secondary metabolites and improvise their properties.

Transcription factor families in plants

Transcription factors (TF) are small proteins involved in the regulation of expression a target gene by binding to its promoter and further modulating the mRNA expression. TFs consist of a DNA-binding domain (DBD), which binds to a specific recognizable sequence in the promoter. TFs are classified into different families based on the characteristic of DBDs (Riechmann et al. 2000). Plants contain both kinds of TFs belongs to all eukaryotic organisms as well as TFs specific to plants only (Hong 2016). *Arabidopsis thaliana* was identified with around 30 TF families (Riechmann et al. 2000). Plant-specific TF families include WRKY, NAC, AP2-ERF, DOF, TCP, YABBY, SBP, GARP, ABI3-VP1 (B3), LFY and EIL (Yamasaki et al. 2013). Other large TF families that are not specific to plants are MADS box, MYB, HB, basic helix–loop–helix (bHLH) and bZIP. These TF families are involved in controlling the growth and development of plants related to the environment. Domain analysis of some families of TFs has been shown in Fig. 1A. TFs like WRKY (Fu et al. 2018; Li et al. 2013; Singh et al. 2017; Yogendra et al. 2017) and MYB (Frerigmann and Gigolashvili 2014; Liu et al. 2016; Wang et al. 2018; Yuan et al. 2014) have been studied for their involvement in the biosynthesis pathway of secondary metabolites. For example, MYB TF regulates biosynthetic pathways of many secondary metabolites, such as GLs (Glucosinolates), flavonoids, HCAAs (hydroxyl cinnamic acid amides) and

Table 1 A list of drugs and bioactive molecules derived from known medicinal plants

Drug/compounds	Plant name	Related disease/disorder/activity
Allicin	<i>Allium sativum</i>	Hypolipidemic
Aloin	<i>Aloe vera</i>	Demulcent, skin diseases
Artemisinin	<i>Artemisia annua</i>	Anti-malarial
Papaverine	<i>Papaver somniferum</i>	Vasodilator
Camptothecin	<i>Camptotheca acuminata</i>	Anticancer
Saponis, Genistein and other flavonoids	<i>Trifolium pratense</i>	Menopausal disorders
Curcumin	<i>Curcuma longa</i>	Antioxidant
Ephedrine	<i>Ephedrae herba</i>	Hypertensive
Ergotamine	<i>Claviceps purpurea</i>	Haemorrhage
Gossypol	<i>Gossypium herbaceum</i>	Contraceptive
Monoterpenes, Sesquiterpenes	<i>Ocimum sanctum</i>	Respiratory diseases, immunomodulatory
Polyphenolics, Tannins	<i>Phyllanthus emblica</i>	Antioxidant
Quinine/Quinidine	<i>Cinchona officinalis</i>	Anti-malarial

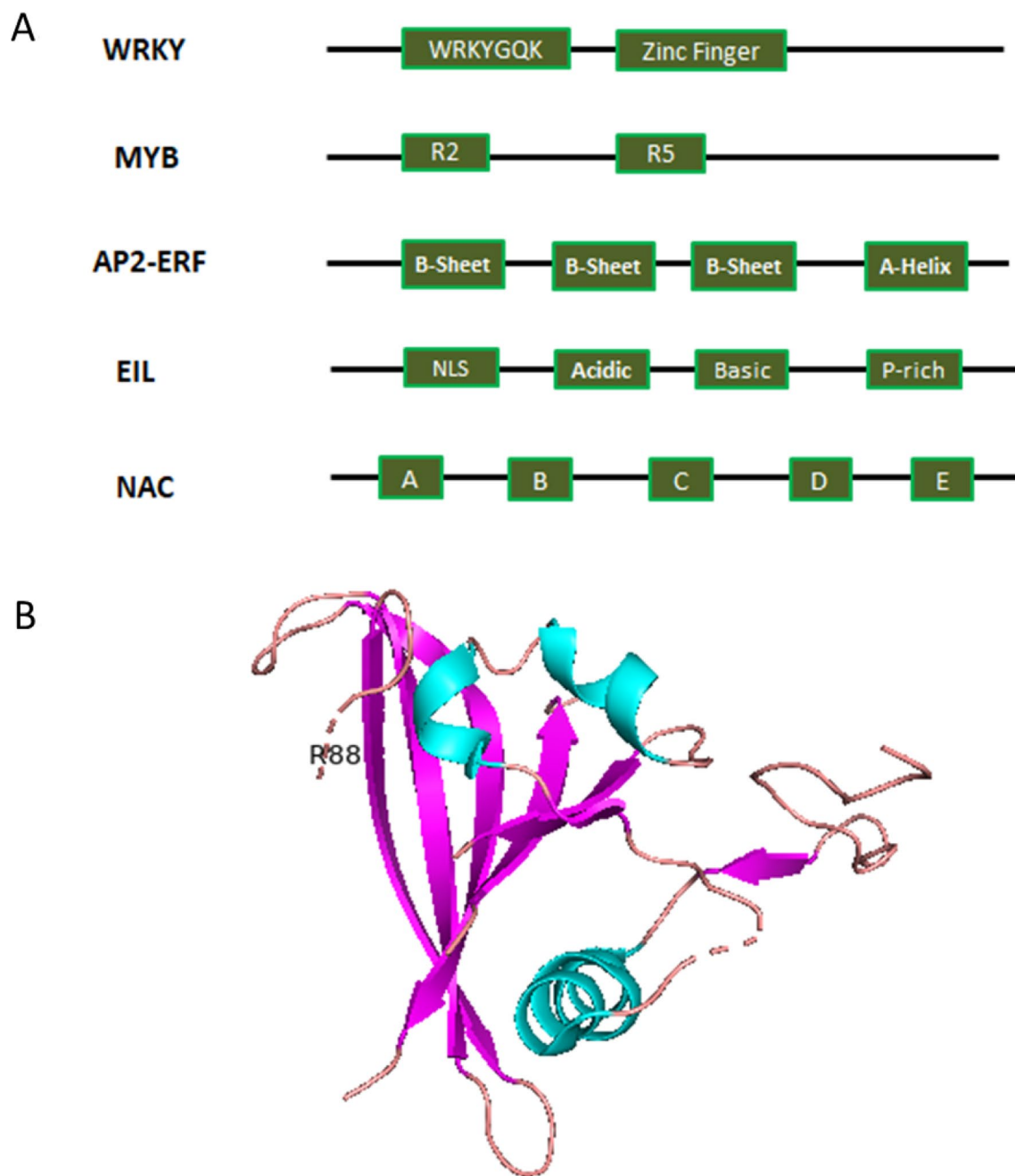


Fig. 1 Domain organization and structure analysis of transcription factors: **A** conserved domain analysis of the transcription factors family's representatives. **B** Cartoon representation of the tertiary structure of ANAC019 (*Arabidopsis thaliana* NAC19; PDB ID: 4DUL). The NAC (NAM, ATAF and CUC) transcription factor structure

majorly contains beta strands, as also presented in the ANAC019 structure. Arginine at 88 position (shown as R88) was found necessary for the binding of DNA along with other required amino acids (Welner et al. 2012)

proanthocyanins. Secondary metabolites can also be produced by stimulus-induced signalling pathways like Jasmonic Acid (JA) or through the activation of several transcription factors, which further induces the genes involved in secondary metabolite production (Fig. 2). However, very few studies have been published with NAC (NAM, ATAF and

CUC) TF as the regulator of secondary metabolite synthesis pathways. Medicinal plants have been reported to contain many sequences of NAC TF with their active involvement in biotic or abiotic stresses. However, it has been reported in very few reports where NAC TF is involved in the biosynthesis pathway of any secondary metabolite or mediating

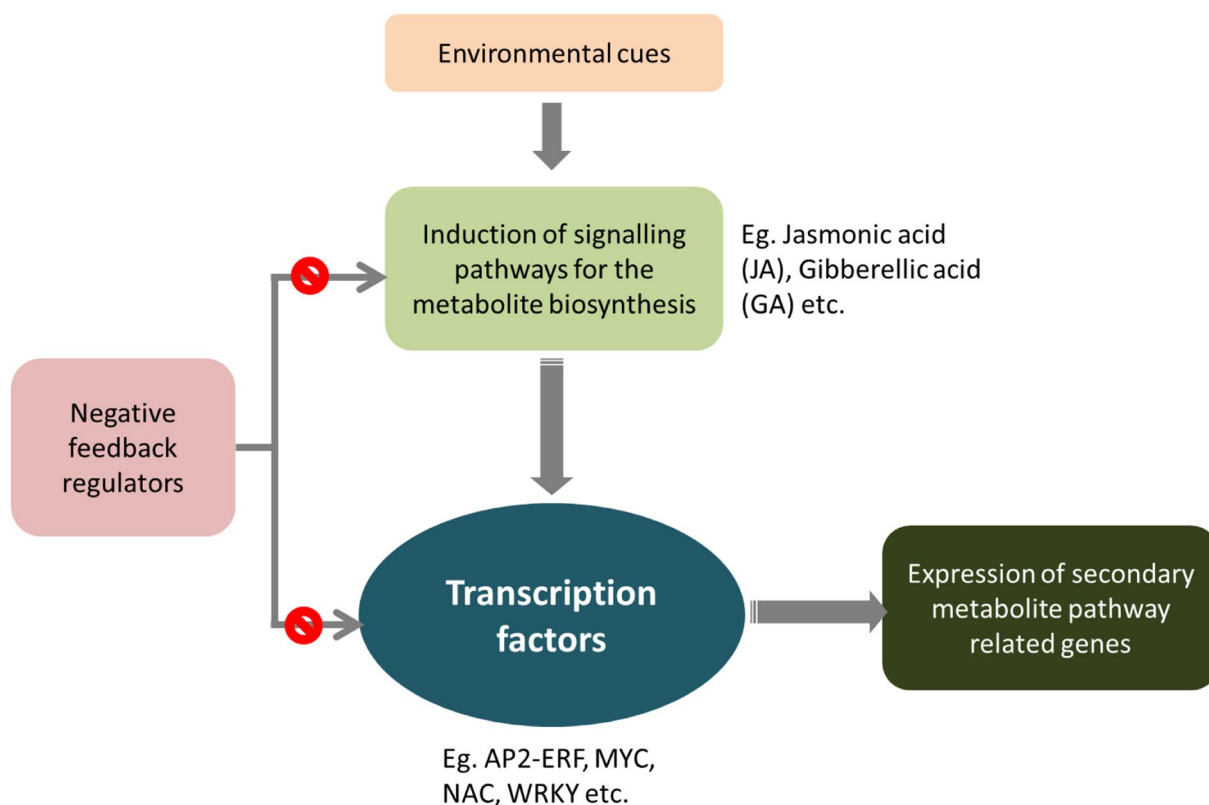


Fig. 2 Transcription factors in the secondary metabolite production through different signalling pathways

signalling pathways, enhancing the medicinal value of a plant.

NAC transcription factor

NAC TFs family is among the many large plant-specific transcription factors families. It is known to be involved in both the development of the plant biotic and abiotic stress regulation (Puranik et al. 2012). NAC gene sequences feature also predicts link throughout the plant evolution, starting from green algae and their adaptation from water to land. *Physcomitrella patens*, moss is an important link for plant adaptation from water to land and a popular model system for the production of plant-based vaccines. It was identified to consist of 31 NAC genes, where NAC genes were analyzed phylogenetically to contribute in the same process (Xu et al. 2014). Similarly, in early land plants like *Selaginella moellendorffii*, NAC genes were identified to link evolutionarily to the late land plants (Jin et al. 2017), *S. moellendorffii* contains 19 NAC genes. Another algal link of adaptation of plants from water to land is *Klebsormidium flaccidum*, where its genome analysis revealed very few NAC genes, i.e., 03 (Mohanta et al. 2020). However, other plants, including early plants, contain a significant

number of NAC genes. This confirms that the NAC genes had been evolved for providing tolerance against multiple stresses to the sessile land plants.

Recently, NAC TF has been reported for its involvement in regulating the plant immune responses hence evoking plant immunity to various pathogens (Yuan et al. 2019). The emerging roles of NAC still have to be explored, but the existing reports support its involvement in several aspects through different pathways (Fig. 3). NAC domain was first identified based on the consensus sequences found in *Petunia* NAM and *Arabidopsis* ATAF1/2 and CUC proteins. NAC TF contains five conserved regions at the N-terminus required for DNA binding with a variable C-terminus containing the transactivation domain (Olsen et al. 2005; Puranik et al. 2012). The tertiary structure of a NAC TF from *Arabidopsis thaliana* presenting a structural composition of conventional NAC TFs has been shown in Fig. 1B. It also exhibits a nuclear localization sequence (NLS) at the N-terminus. NAC has been identified to contain a large number of sequences in many plants, mainly in land plants. *Brassica napus* consist of the maximum number of NAC TF sequences, i.e., 410 NAC TFs, whereas the pteridophyte, *Marchantia polymorpha*, consists of the lowest number, i.e., 9 NAC TFs (Mohanta et al. 2020). Other than this, NAC was also identified to be present in algae and bacteria.

Fig. 3 Role of NAC (NAM, ATAF and CUC) transcription factor genes in plants explored yet

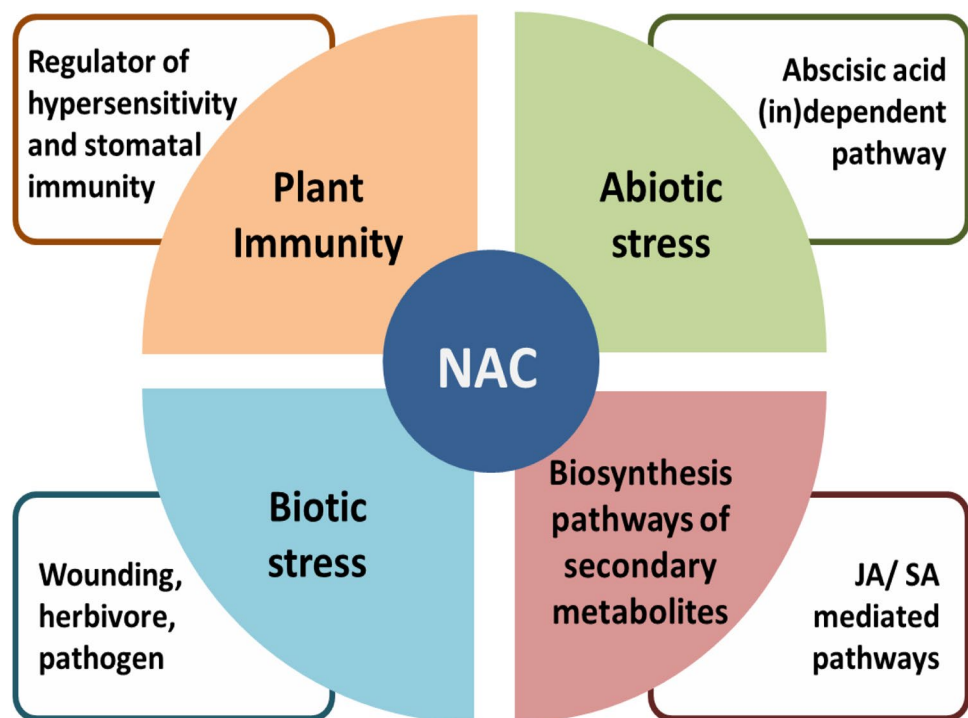


Table 2 Number of NAC (NAM, ATAF and CUC) transcription factor genes in plants

Plant species	Number of NAC TFs	Class
<i>Arabidopsis thaliana</i>	117	Dicot
<i>Oryza sativa</i>	151	Monocot
<i>Zea mays</i>	130	Monocot
<i>Brassica napus</i>	410	Dicot
<i>Brassica rapa</i> *	256	Dicot
<i>Eucalyptus camaldulensis</i>	200	Dicot
<i>Artemisia annua</i> *	28	Dicot
<i>Glycine max</i>	180	Dicot
<i>Gossypium hirsutum</i>	306	Dicot
<i>Nicotiana tabacum</i> *	280	Dicot
<i>Ocimum tenuiflorum</i> *	110	Dicot
<i>Populus trichocarpa</i>	169	Dicot
<i>Sesamum indicum</i>	105	Dicot
<i>Triticum aestivum</i>	265	Monocot
<i>Spinacia oleracea</i>	45	Dicot
<i>Trachyspermum ammi</i> *	68	Dicot
<i>Trifolium pratense</i> *	97	Dicot
<i>Pinus taeda</i>	31	Gymnosperm
<i>Selaginella moellendorffii</i>	22	Pteridophyte
<i>Physcomitrella patens</i>	33	Bryophyte
<i>Sphagnum fallax</i>	26	Bryophyte
<i>Klebsormidium flaccidum</i>	3	Algae

*Medicinal plants

Genome-wide analysis of NAC proteins sequence reveals its presence in 160 species (18774 NAC sequences). Table 2 contains a list of plants containing NAC TF genes along with the medicinal plants (Mohanta et al. 2020). The medicinal plants in the table are marked with asterisk symbol (Al-Lahham et al. 2020; Ariey et al. 2014; Bairwa et al. 2012; Hong and Kim 2008; Kolodziejczyk-Czepas 2012; Pattanayak et al. 2010). Monocot plants were found to contain NAC TFs in higher numbers as compared to dicots.

NAC TFs: regulation and expression

The NAC TFs protein regulation is shown in Fig. 4. NAC TFs transcriptional activation processes are associated with the C-terminal of NAC protein, where the N-terminal NAC domain entails DNA binding (Olsen et al. 2005). These NAC transcription factors play an important role in stress tolerance in plants. Transcription factors like DREB (Dehydration-responsive element-binding protein) and AREB (ABA-responsive element-binding protein) become stress induced during modulation of transcriptional activity of NADs. These TFs contain binding sites for NAC and when NAC binds with them they become stress-induced (Mochida et al. 2009). During transcriptional regulation, the pursuit of a gene can be controlled by binding particular TFs with its regulatory region in the promoter. In NAC TFs gene at promoter region, numerous stress-responsive elements are present and they act as stress responsive. These elements

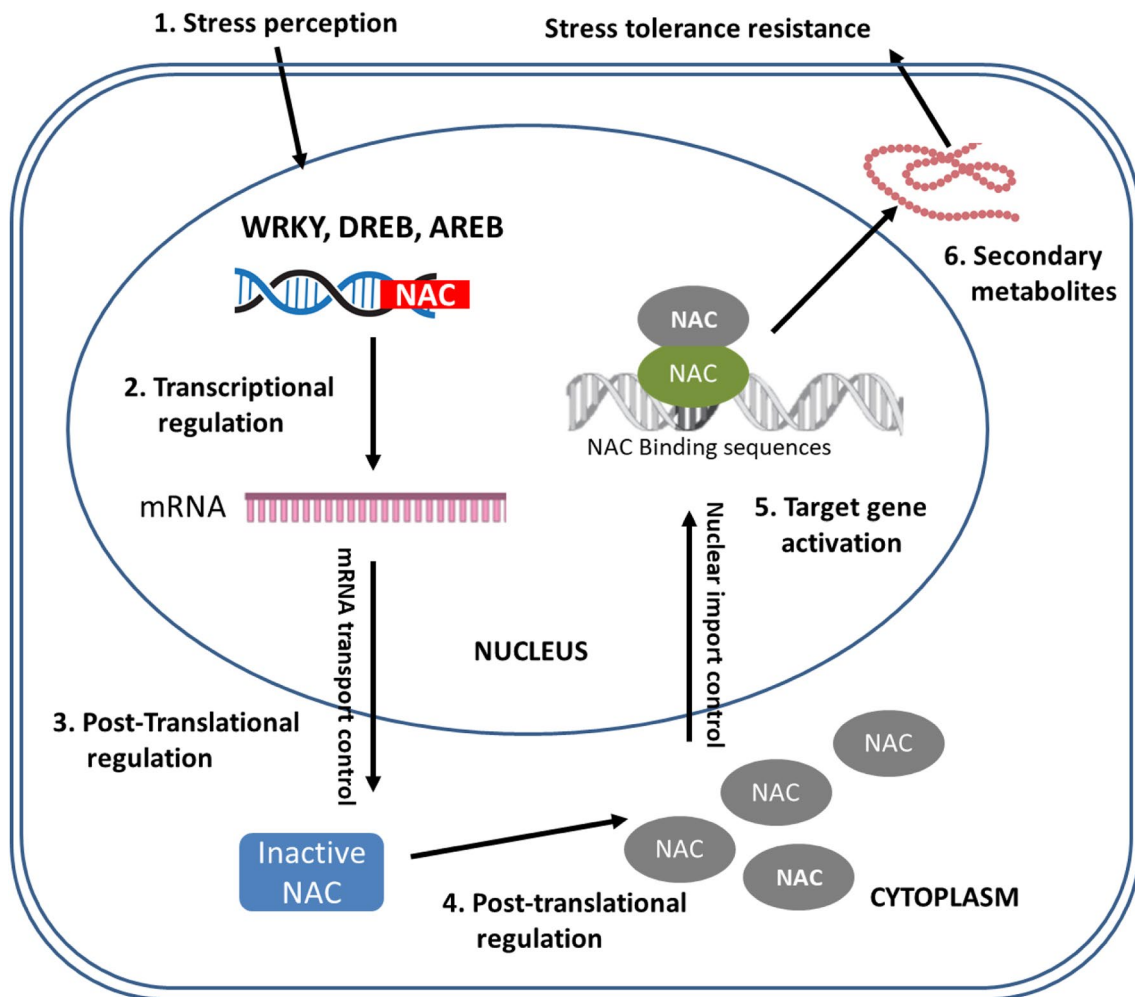


Fig. 4 Schematic diagram representing the regulation of NAC (NAM, ATAF and CUC) transcription factors under stress perception; the different major steps undergone are shown pointwise. transcription factors such as DREB (Dehydration-responsive element-binding protein) and AREB (ABA-responsive element-binding protein) may bind to the promoter region of NAC gene under stress perception leading

further to its transcription. Subsequent premature synthesized mRNA then goes through multiple steps resulting into NAC proteins synthesis. Further, after post-translational step and other additional modifications, these processed dimerized-NAC transcriptional factors act as a PR (pathogenesis-related) gene promoter by binding with NAC-binding sequences

comprise LTREs, MYB and DREB. The DREB persuades the expression of NAC transcription factors like *OcNAC3* and *SNAC1* during abiotic stress. MYB and NYC in the promoter region persuade the expression of other important NAC protein, such as ONACO45 (Nakashima et al. 2012; Takasaki et al. 2010; Zheng et al. 2009).

Regulation of NAC transcriptional factor miRNA164 is entailed with it particularly during stress. This miRNA164 acts as a regulator for post-transcriptional regulation because it is a micro-RNA and micro-RNAs help in the cleavage of genes during the post-transcriptional phase (Khraiwesh et al. 2012). During post-translational regulation of NAC transcription factors, phosphorylation of ATAF1 with the help of SnRK1 occurs, this attunes subcellular localization of ATAF1 and this reaction regulates the DNA-binding ability

of ATAF1. However, this interaction ATAF1 becomes a prey for proteasomal degradation during the interaction between SnRK1 and E3-Like ligases (Kaneda et al. 2009; Kleinow et al. 2009; Li et al. 2011).

For conserving recognition sequences for NAC transcription factors, which are stress responsive, numerous NAC transcription factors bind with NACRS (NAC recognition sequence) and here these NAC transcription factors act as PR gene promoters. Conditions like dwarfism and late flowering are seen in some transgenic plants and these conditions arise due to constitutive overexpression of NACs, but these conditions can be controlled to some extent using stress inducible promoters, such as *OsNAC6* or *RCc3*. NAC transcription factors on binding with promoters of various subsequent genes administrate their transcription. During

transcriptional regulation of NACs, the ABA responsiveness is administered by the ABRE sequence present in *OsNAC5* and *OsNAC6*. The transcription of NAC genes is swayed by subsequent transcription factors, such as DRFBs or ABREs and these subsequent transcription factors bind with cis-regulatory elements (Jeong et al. 2010; Nakashima et al. 2007).

NAC TFs in medicinal plants

NAC TF genes have been reported to be present in plants with medicinal properties (Jensen et al. 2010). However, their detailed characterization and functional significance in relation to medicinal plants are minimal. The function of NAC TFs in regulating plant abiotic and biotic stresses has been studied from the last few years. However, limited reports have shown the involvement of NAC TFs in regulating secondary metabolites (Duan et al. 2017; Mahmood et al. 2016). Secondary metabolite production is regulated at the transcription level by several transcription factors (Patra et al. 2013). Here, we have highlighted the role of NAC TFs in response to various biotic and abiotic stresses of some medicinal plants.

Populus trichocarpa

P. trichocarpa, also known as western balsam poplar, is a fast-growing deciduous tree which belongs to the Salicaceae (willow) family is a typical model tree. It is well utilized for its herbal properties, although rarely reported scientifically. The leaf buds and bark consist of major phytochemicals that exhibit antiseptic and expectorant properties. They contain salicin, a glycoside that decomposes into salicylic acid (aspirin) in the body. Leaf buds are antiscorbutic, antiseptic, diuretic and expectorant in nature, which is implemented for the treatment of bronchitis and respiratory tract infections (Database searched at Pearl and Darling 1971; Xu et al. 2014)). Bark exhibits anti-inflammatory properties, especially for treating rheumatic fever and relieving the pain during menstruation (Turner and Hebda 1990).

Populus have been identified with 169 genes of NAC TF (Mohanta et al. 2020). However, NAC TF has not been functionally characterized in *Populus* yet. The thickening of secondary walls of xylem cells and deposition of lignocellulose is focused on the large biomass production of wood from plants like *Populus*. Several transcription factors like MYB and NAC play a role in these secondary wall biosynthesis pathways. In *Arabidopsis thaliana*, a group of genes belonging to NAC TF, including NST1, NST2, VND6, SND1 and VND7, was characterized for their biosynthesis part of the secondary wall (Mitsuda et al. 2007; Yamaguchi et al. 2008; Zhong et al. 2007). Zhong et al. (2010) reported the functional characterization of 6 NAC genes in *P. trichocarpa*,

which are collectively called *PtrWND*s (Wood-Associated NAC Domain Transcription Factors). Among the 6 NAC genes, *WND2B* and *WND6B* were seen to complement the defects of secondary walls in their *Arabidopsis* double mutant, *snd1/nst1*. Moreover, their overexpression in *Arabidopsis* induced external deposition of secondary walls. These results indicated the two genes, *WND2B* and *WND6B*, to be the orthologues of *Arabidopsis SND1* and are master switches of secondary wall biosynthesis in *Populus* during wood formation (Zhong et al. 2010). Comprehensive analysis of *Populus* NAC genes was also performed with extensive computational analysis, gene expression using microarray data and confirmed the expression of selected NAC genes by qRT-PCR (Hu et al. 2010). This study phylogenetically divided the NAC genes of *Populus* into 18 subfamilies and suggested sub-functionalities, which gives a foundation for functional characterization of NAC genes in the future. Three more *Populus* NAC genes, identified as *PtrNAC006*, *PtrNAC007* and *PtrNAC120*, were reported for their presence in drought-related stress response, promoters of which were further activated by AREB transcription factor (Li et al. 2019). In different species of *Populus*, RNA sequencing analysis under salt stress showed induction in the expression of 289 genes (Wang et al. 2019), among these, two genes, *PtNAC024* and *PtNAC182*, were found correlated to the other 63 differentially expressed genes.

Artemisia annua

A. annua is also known as sweet wormwood, belongs to the Asteraceae family and used as a medicinal plant in China for a long time. It contains a compound called artemisinin with the potential for the production of antimalarial drugs. Artemisinin is the secondary metabolite harboured by *A. annua*. It is sesquiterpene lactone endoperoxide, which is widely used as an antimalarial drug (Yu et al. 2012). Artemisinin is seen to be effective against *Plasmodium falciparum* which is chloroquine- and quinine resistant and other parasites causing malaria (Ariey et al. 2014). It also possesses antiviral (Romero et al. 2006), anticancer (Efferth 2006) and antischistosomal activities (Utzinger et al. 2007) besides their antimalarial activity.

A. annua is the only available natural source for artemisinin with low yield. Researchers tried to improve artemisinin production by identifying and modifying the components involved in its biosynthetic pathway. Jasmonic acid (JA) was reported to elicit the production of artemisinin and other gene expression in *A. annua* through inducing JA-responsive transcription factors and others (Caretto et al. 2011). *A. annua* genome was found to contain 28 NAC TF genes. One NAC gene of *A. annua*, *AaNAC1*, has been overexpressed and characterized (Lv et al. 2016). *AaNAC1* expresses in the stress conditions like dehydration, cold and

others, indicating its role in stress regulation. It was found to be localized in the nucleus. Overexpression of *AaNAC1* in *A. annua* revealed an increment of around 79% and 150% in the content of artemisinin and dihydroartemisinic acid, respectively. The expression levels of genes related to artemisinin biosynthetic pathway like amorpha-4,11-diene synthase (ADS) and others, some of which were also regulated by JA, was found to be increased during overexpression of *AaNAC1*. Transgenic *A. annua* also exhibits resistance to *Botrytis cinerea* along with tolerance to drought. This overexpression study suggested the potential use of *AaNAC1* in the improvement of the artemisinin content through development of transgenic plants. Other than NAC, a SPL transcription factor, *AaSPL2*, was also reported to be involved in the increment of artemisinin content through the JA signalling pathway (Lv et al. 2019) further studies will be able to reveal the possible involvement of other transcription factors like NAC in these JA signalling-mediated biosynthetic pathways.

Trachyspermum ammi (Ajwain)

Ajwain (*T. ammi*) is an aromatic and herbaceous plant of the family Apiaceae. *T. ammi* seeds are widely used in diet and traditional medicine. Seeds of this plant contain oil (2–4.4%), known as ajwain oil. It exhibits antimicrobial, fungicidal and anti-aggregatory effects on humans. *Ajwain* fruit possesses stimulant, antispasmodic and carminative properties. It has great uses as the remedial agent for flatulence, atonic dyspepsia and diarrhoea. A major component of the ajwain oil is thymol (35% to 60%). It is a monoterpene derivative of isoprene hydrocarbone (2-methyl-1, 3-butadiene). It is formed by the combination of two or more isoprenes. Thymol is used for lack of appetite, bronchial problems and gastrointestinal ailments. It is also used in toothpaste and perfumery. Thymol has multiple biological activities: antifungal (Mahmoud 1994), antiviral (Hussein et al. 2000), anti-inflammatory (Azuma et al. 1986) along with antioxidant (Aeschbach et al. 1994). Other constituents of the ajwain seed are fiber, tannins, carbohydrates, glycosides, fat, flavone, protein, saponins and several minerals containing calcium, iron, nicotinic acid and phosphorus. Therapeutic uses of *T. ammi* include expectorant, carminative, antiseptic, amoebiasis and antimicrobial (Bairwa et al. 2012).

Transcriptome analysis of *T. ammi* inflorescence tissues revealed the presence of transcription factors from almost all families with NAC TFs accounting for around 6% of the total (Amiripour et al. 2019). It was the second prevalent TF family in the *T. ammi* transcriptome TF dataset. Another comparative transcriptome analysis of the inflorescence of *T. ammi* ecotypes was carried out to identify the genes involved, specifically in the biosynthesis of thymol. The differentially expressed unigenes, comprising of the genes encoding hydrogenases, transcription factors

and cytochrome P450, suggested being associated with the diversity of terpenoids in *T. ammi* plants (Soltani Howyze et al. 2018). Datasets revealed the differential expression of 73 unigenes that belongs to 24 TFs families, including bZIP, GATA, bHLH, C3H, WRKY, NAC and MYB families (Soltani Howyze et al. 2018). NAC genes were among the highest differentially expressing TF genes. The high expression of NAC genes in the inflorescence of *T. ammi* indicates a role for NAC TF in the terpenes biosynthesis (Soltani Howyze et al. 2018) and hence can be used to enhance the thymol production through transgenic plant approach.

Nicotiana tabacum

N. tabacum is a perennial herbaceous plant and belongs to the family of Solanaceae. Its leaves and galls are known to possess the potential for traditional remedies. *N. tabacum* is a research model plant with high economic value worldwide. The plant galls extract is used as a traditional medicine in various pathologies-related treatments. The tobacco leaves are applied externally to treat rheumatic swelling, painful piles, skin diseases and stings. The main secondary metabolite found in *Nicotiana* is nicotine, a tobacco-specific alkaloid. Nicotine biosynthesis involves the formation and coupling of both pyrrolidine ring and pyridine ring formation. Synthesis of nicotine takes place in tobacco roots, then transported to the leaves through the xylem. The leaves are diuretic, antispasmodic, emetic, expectorant, narcotic, irritant, sedative and sialagogue (Database search at, (Xu et al. 2014)). Nicotine drug from the tobacco plant that stimulates nicotinic acetylcholine receptors suggested that it can be a therapeutic intervention for Parkinson's disease (Quik et al. 2008).

Regulation of nicotine biosynthesis involves a number of transcription factors as well as JAs, the latter is known as a positive regulator (Dewey and Xie 2013; Kato et al. 2014; Wang and Bennetzen 2015). *N. tabacum* in total 280 NAC TFs has been reported (Mohanta et al. 2020). Some of the TFs, such as *NtNACs* have been characterized functionally. *NtNAC2* gene was found to be with upregulated expression during drought stress indicating its important role in controlling drought stress in plants (Xu et al. 2018). The overexpression of *NtNAC2* showed that it increases the photosynthetic rate. The antioxidant enzymes activities, such as peroxidase and superoxide dismutase were found to be increased the *NtNAC2* overexpression in Tobacco. These results indicated that the *NtNAC2* functions positively in regulating drought stress. In another study, *NtNAC2* has also been reported to be a salt-inducible gene by Han et al. 2015). Tissue-specific expression of *NtNAC2* revealed its expression in roots, stems and flowers. The topping of *Tobacco* plants resulted in the increased biosynthesis of nicotine in the roots. Being a topping responsive gene (Qi et al. 2012),

NtNAC-R1 transcription factor was suggested to communicate this signal. An increase in *NtNAC-R1* upregulates the expression of key enzyme of the nicotine biosynthetic pathway, *PMT*, proved by both transgenic tobacco with miRNA silenced and overexpressed of *NTNAC-R1*. In conclusion, the upregulated expression of *NtNAC-R1* in *tobacco* results in an increase in lateral roots and nicotine content, along with crosstalk with JA and auxin signalling pathway (Fu et al. 2013). Detailed study of other NAC genes in *tobacco* will be able to suggest their role in other biologically and economically important secondary metabolites biosynthesis pathway.

Andrographis paniculata

A. paniculata is commonly known as creat or chireta, belongs to the family Acanthaceae with many medicinal properties. Its aerial parts and roots are used as a traditional medicine in several countries. *A. paniculata* is favoured medicinal plants, traditionally used to treat diseases like diabetes, cancer, leprosy, ulcer, high blood pressure, bronchitis, skin diseases, flatulence, colic, dysentery, influenza and malaria for centuries. Several phytochemical constituents of *A. paniculata* include flavonoids, diterpenes, noriridoides, xanthenes and other miscellaneous compounds (Tajidin et al. 2019). Among diterpenes, Andrographolide is the most prominent and pharmacologically important (Pan-zica 2016). These compounds and plant extracts have been identified for their cytotoxicity, antimicrobial, antioxidant, anti-inflammatory, immune-stimulant, anti-infective, anti-diabetic, hepato-renal protective, anti-angiogenic, insecticidal activities and liver enzymes modulation (Okhwarobo et al. 2014). The recent reports suggested the potential of *A. paniculata* leaf extract as a powerful anticancer agent (Paul et al. 2019). Tumour hypoxia leads to angiogenesis through hypoxia-inducible factor-1 α (HIF-1 α) inducible vascular endothelial growth factor (VEGF) signalling, creating tumour microenvironment inhibiting drug delivery to these tissues. *A. paniculata* leaf extracts significantly downregulate the expressions of HIF-1 α and VEGF, both at the transcriptional and translational levels. The molecular mechanism behind this was suggested to involve transcription factors involved in the hypoxia signalling cascade, whose altered expressions leads to the downregulation of HIF-1 α (Paul et al. 2019).

A. paniculata leaf transcriptome analysis revealed the presence of around 146 different transcripts for the terpenoids biosynthesis related enzymes. Among these, 35 transcripts possessed motifs for the terpene synthases. 6767 unique transcripts were identified as encoding plant-specific and nonspecific transcription factors from 97 transcription factor families (Cherukupalli et al. 2016). Most of them represented AP2ERE BP family, followed by WRKY, then NAC and B3-Domain, etc. NAC transcription factors are

still not studied in details for *A. paniculata*. Among the terpenoids, several methods have been studied to stimulate the production of andrographolide large amounts (Pan-zica 2016). Several synthetic inducers like cytokinin-1-(2-chloro-4-pyridyl)-3-phenylurea (CPPU) have been reported to increase the yield of andrographolide. Methyl jasmonate (MeJA) has also been used as an elicitor for the overproduction of andrographolide (Sharma et al. 2015). A single NAC transcription factor, ApNAC1, has been characterized in *A. paniculata* (Wang et al. 2017), this is localized in the nucleus and predominantly expresses in the leaves. Its expression also increased on MeJA treatment, which indicates its involvement in the biosynthesis of andrographolide. The detailed molecular study would reveal its direct role in the biosynthesis of andrographolide and other medically important secondary metabolites in *A. paniculata*.

Ocimum tenuiflorum

O. tenuiflorum or *O. sanctum*, an Indian native and commonly called Holy Basil or *Tulsi*, is a perennial plant belonging to the family of Lamiaceae. *Tulsi* is pre-eminent among all the herbs used for Ayurvedic medicines. *Ocimum* is a widely known genus for its medicinal, ethnobotanical and aromatic properties. These properties are attributed to many terpenoids and phenylpropanoid compounds produced by this plant species. Traditionally, different parts like leaves, flower, root, stem, seeds, and also whole plant, of *O. tenuiflorum* have been used to treat malaria, bronchitis, skin disease, dysentery, arthritis, insect bites and more. It has also been indicated to possess anticancer, anti-diabetic, antifungal, cardioprotective, antimicrobial, adaptogenic and antispasmodic actions (Manaharan et al. 2014; Pattanayak et al. 2010; Yamani et al. 2016). Leaves and inflorescence extracts of *Tulsi*, available as an oil formulation, suggested having numerous valuable properties, such as analgesics, expectorants, stress reducers and anti-emetics, inflammation relievers, anti-asthmatic and antipyretics; hepatoprotective, hypoglycemic, hypolipidemic, hypotensive and immunomodulatory agents (Singh et al. 2010; Yamani et al. 2016). Eugenol (1-hydroxy-2-methoxy-4-allylbenzene) is the most active constituent of *O. tenuiflorum* and harbours great therapeutic potentials (Pattanayak et al. 2010). Eugenol has a known pharmacological effect on the immune system, cardiovascular system, central nervous system, urinary and reproductive system (Prakash and Gupta 2005).

De novo transcriptome sequencing in *O. sanctum* and *O. basilicum* revealed the presence of around 5.9% transcripts to encode for approximately 40 TF families (Rastogi et al. 2014). Examples of TF families include bHLH, MYB, AP2/ERF/DREB, NAC, WRKY, bZIP, C2H2 and many others. *O. tenuiflorum* specifically has been identified to contain 110 NAC TF genes (Mohanta et al. 2020). In the family of

Lamiaceae, for example, *Perilla frutescens* and *Salvia miltiorrhiza*, bHLH TFs are reported to be involved in biosynthesis pathway of phenyl propanoid (Gong et al. 1999; Wang et al. 2011). However, TFs involvement in the biosynthesis of secondary metabolites in *O. tenuiflorum* is still not studied in detail. A detailed study and analysis of the terpenoid and phenylpropanoid biosynthetic pathways would be able to reveal the functional significance and use of NAC TFs in the overproduction of these valuable products. Various TF families have been reported to be affected by abiotic stresses like cold, flood, drought and salinity of *Ocimum* (Rastogi et al. 2019). NAC TF was identified to be the most affected TF family, including WRKY, MYB, bHLH and ERF. WRKY, MYB and NAC have been previously known to be involved in abiotic stress control in rice (Gujjar et al. 2014).

Overexpression and genetic engineering of NAC genes in plants

Discovery of plant NAC genes through whole genome and expression analysis revealed a vast distribution of NAC genes in plants. Their molecular and functional characterization is still limited, leaving a broad range of unexplored research related to NAC roles in plants. Till now, NAC TFs have been explored through expression profiling analysis in plants for their involvement in the regulation of multiple biotic and abiotic stresses. According to several reports, the overexpression of specific NAC genes in different plants can lead to the development of transgenic plants with stress tolerance properties. The recent advanced gene-editing tools and transformations can make it easier and economical to produce plants phenotypically expressing NAC-associated traits (Fig. 5). The overexpression of the NAC gene from *Lepidium latifolium* in tobacco induces stress tolerance and enhances biomass production (Grover et al. 2014). *ANAC019*, *ANAC055* and *ANAC072* genes overexpression in *Arabidopsis* provide drought tolerance in comparison to wild type (Bu et al. 2008). Transgenic *Arabidopsis* with overexpression of *SINAC1* of *Suaeda liaotungensis* was shown to possess enhanced tolerance to drought, cold and salt stresses (Li et al. 2014). *CaNAC035*, from pepper (*Capsicum annuum*), silencing caused more damage in response to cold, NaCl and mannitol treatment, whereas its overexpression in *Arabidopsis* plant confers a higher germination rate with fresh weight after stress treatment (Zhang et al. 2020). Overexpression of *OsNAC6* transcription factor enhances salt and drought tolerance in rice (Rachmat et al. 2014). Some examples include the improvement of crop plants against abiotic stresses; thereby, leading to increased production. Transgenic *Glycine max* overexpressing *GmNAC11* gene showed enhanced growth of lateral roots under salt stress (Hao et al. 2011). The overexpression of *TaNAC69*, driven

by a drought-inducible promoter (HvDhn4s) in transgenic wheat, improves water-use efficiency and dehydration tolerance (Xue et al. 2011). However, the grain weight was observed to be significantly reduced in these constitutively expressed *TaNAC69-1* lines. Overexpression studies of NAC specific to medicinal plants have not been done in the plant itself to prepare the transgenic or to improve the medicinal crop. The studies are still focused to the functional characterization of the NAC genes from medicinal plants that too in a few plants only, such as *A. annua* (Lv et al. 2016).

Many transgenics obtained with NAC genes have delivered promising results; however, there are some limitations, considering which there might be a need to manipulate the NAC genes carefully to overcome the problems. For instance, the transgenic plants with NAC genes' overexpression may sometimes respond antagonistically to various stresses. For example, *Arabidopsis* transgenic overexpressing ATAF1 with drought tolerance was highly sensitive to oxidative stress, high salt and necrotrophic fungus (Wu et al. 2009) and overexpression of *ANAC019* and *ANAC055*, along with the increment of drought tolerance, also decreased resistance to *B. cinerea* (Bu et al. 2008; Fujita et al. 2004). The NAC gene sequences responsible for these types of negative impacts need to be identified and engineered accordingly to avoid the adverse impacts. In case of medicinal plants, this type of studies has not been conducted yet other than their functional analysis in few cases. Further, a better understanding of the molecular and regulation mechanism may lead towards genetic engineering, leading to the increased secondary metabolites production, better stress tolerances and immunity of the medicinal plants.

Conclusion and future prospective

Medicinal plants hold great potential to substitute the current pool of synthetic drugs with biopharmaceuticals, including the production of biocompatible drugs and invaluable ayurvedic compounds in an economical way. Biosynthetic pathways of these metabolites involve a set of transcription factors that regulates the secondary metabolite production. One of the families of the transcription factor, NAC comprises a measurable fraction of plant genomes has not yet been studied in detail for medicinal plants. The structural and functional characterization of NAC TF proteins are the emerging research field for using NAC TF to produce gene edited or transgenic plants. The family of NAC TF has been found to be involved in the biotic and abiotic stresses of plants. Several reports also revealed the involvement of NAC TF in the regulation of the biosynthesis pathway of secondary metabolites in plants. Overexpression of the NAC TF gene in these plants would lead to produce the required metabolites in an economical way. In the case of medicinal plants, where

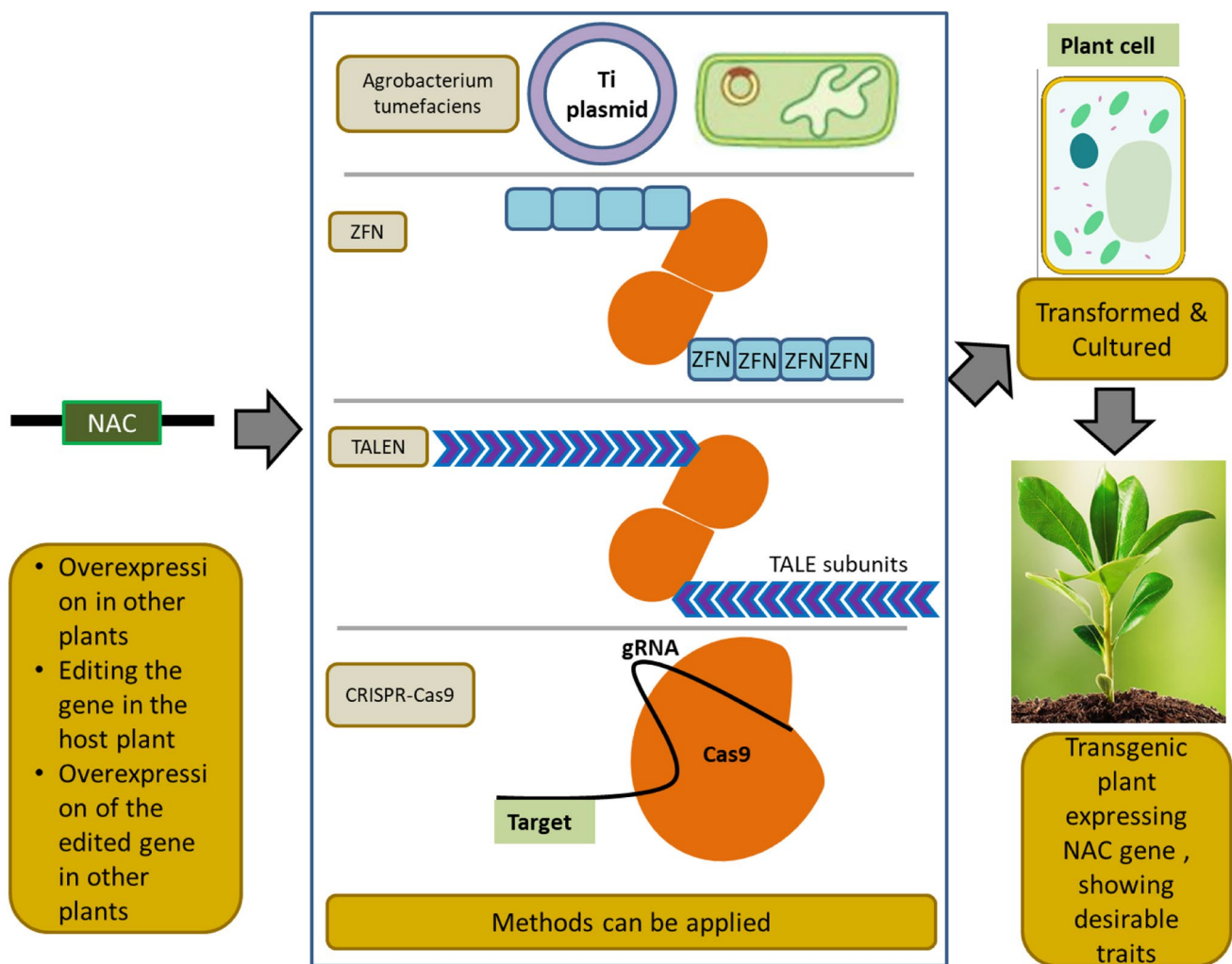


Fig. 5 Overexpression of NAC (NAM, ATAF and CUC) gene in plants: simplified steps of NAC gene over expression in plant cell along with details of available gene editing tools like conventional Ti DNA plasmid approach and advanced like zinc finger nuclease and TALEN (transcription activator-like effector nucleases). The most

recent is CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 gene editing tool that made the genetic engineering and overexpression of NAC gene into the plants, simpler and economical than earlier

secondary metabolites are of pharmacological importance, but their lower and expensive production is a bottleneck for pharmaceutical companies can be easily addressed by using above-mentioned approach. Engineering of NAC TF genes to regulate stress-responsive or secondary metabolite pathways will produce stress-tolerant or economically metabolite overexpressing transgenic plants. In conclusion, NAC TF genes harbour the potential to improve the quantitative production of biopharmaceuticals in plants, which must be explored in the future.

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Data availability The data supporting this review are from previously reported studies and datasets, which have been cited.

Declarations

Conflict of interest The authors declare that they have no conflict of interest in the publication.

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