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Himalayan diversity: An ethnopoetic medicinal plant

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Abstract
Picrorhiza kurroa Royle ex Benth is an established medicinal plant. It is widespread from subalpine to alpine region of India as well. Medicinal properties due to the presence of iridoid glycosides, phenolic glycosides, and cucurbitacin glycosides are well established. Depletion natural or otherwise of this plant are due to described reasons in this communication including elicitors and biogenesis of secondary metabolites. Since, leads are available for various effects and the fact of combinatorial molecules and propagational strategies makes its further value. Cellular effects during cultures in cell lines of this plant using elicitors of microenvironment of certain cell lines such as hyperhydricities, flowering, metabolites promoting for various molecular behaviours in plants during regime(s) of climate change.

Keywords: Bioresources, ethnobotany, ethnopharmcology, metabolomics, pharmacogenomics

Introduction
The genus *Picrorhiza* is the member of established having high medicinal properties. This plant comprises two species, *Picrorhiza kurroa* Royle ex Benth and *Picrorhiza scrophulariiflora* Pennel both have biological effects due to *P. kurroa* contain pikuroside, veronicoside, phenol glycosides, cucurbitacin glycosides and 4-hydroxyl-3-methoxy-acetophenone, whereas *P. scrophulariiflora* contains cyclopentanoid monoterpenes, caffeoyl glycosides, phenylethanoid glycoside and plantamajoside.
The particular species are being observed in the Himalaya. In India also it is naturally distributed from sub-alpine to alpine regions of North-Western Himalayan range and North-Eastern between 3000-5300m. Initially *P. kurroa* medicinal properties are due to the iridoid glycosides like picrosides I, II, III, V, kutkosides and other active constituents viz. Apocyanin, drosin and cucurbitacins later on [1].
Present plant material under our observation for last 5-6 years under greenhouse appears difficulty based on our materiological later.

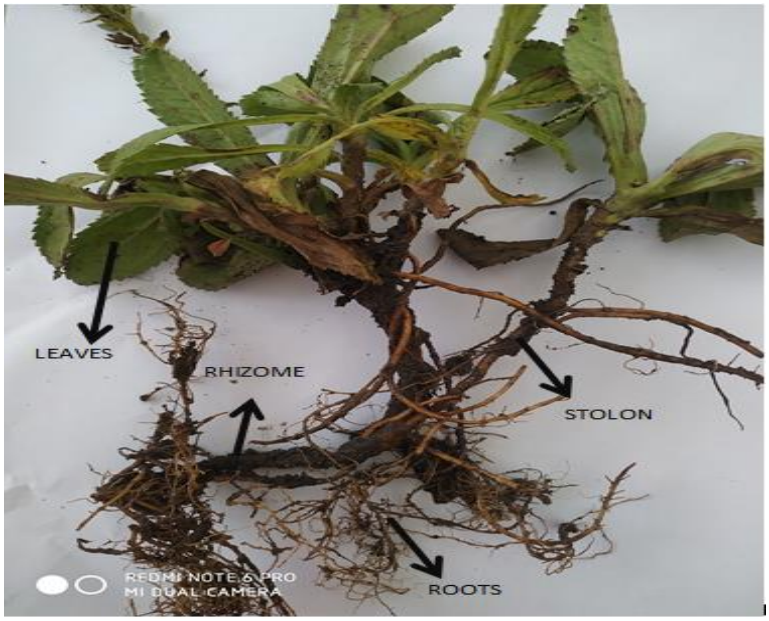


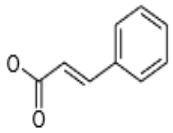
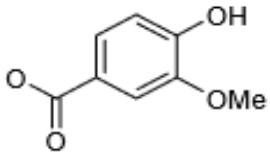
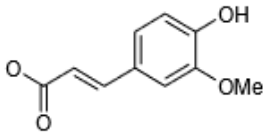
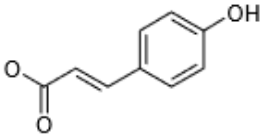
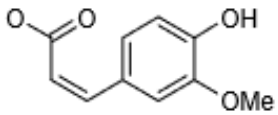
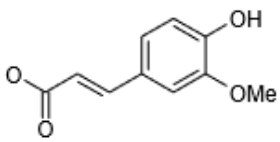
Fig 1: Figure showing parts of *Picrorhiza kurroa*

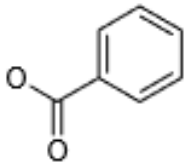
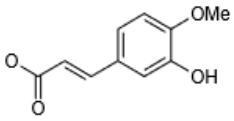
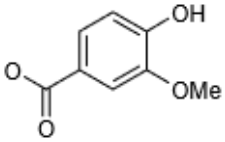
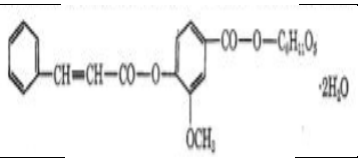
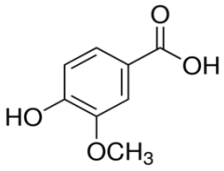
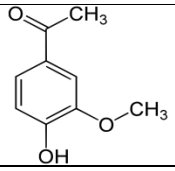
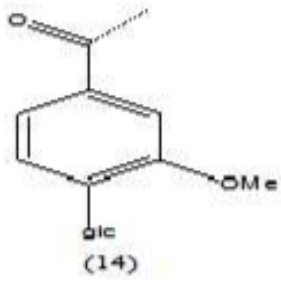
***Picrorhiza kurroa* Royle ex Benth**

Picrorhiza is a small perennial herb which is the member of family Scrophulariaceae. This family was placed in the order scrophulariales, Asteridae (subclass) and Dicotyledonae class of Angiospermae as per the taxonomical system of Cronquist. In 1876, *Picrorhiza* genus was described in ''Genera Plantarum''by Bentham and Hooker. The genus *Picrorhiza* was thought to be monotypic earlier, consists of *P. kurroa* as only one species. Later a second species, was identified by Pennell i.e *P. scrophulariiflora* (also can be written as ''*P. scrophulariaeflora*'') based on information in ''Flora of British India'' written by Hooker [2]. A drawing was published by Royle on 24 August 1835 in his ''Illustrations of Botany'' [3], where for the first time the species *P. kurroa* and genus *Picrorhiza* appeared. On 17 Nov. 1835 Bentham illustrated this genus and species in ''ScrophularineaeIndicae'' [4]**.** ''*Picrorhiza kurroa''* name was stated by Bentham, It might be due to misspelling of the species name written in Royle's publication or he tried to correct the species name **[5].** Bentham given the accepted name of the species ''*Picrorhiza kurroa* Bentham'' or ''*Picrorhiza kurroa* Royle ex Bentham''. Nevertheless, any illustration with analysis which had been published before 1 Jan. 1908 is considered as valid publication according to ICBN (International Code of Botanical Nomenclature) article number 42, rather than any written description [6]**.** Hence, the right species name is ''*Picrorhiza kurroa* Royle'' [7]. Generic name has been derived from bitter root, which is used in traditional medicine practices [5]. In Greek, ''picros'' means bitter, while ''rhiza'' means root. Karu (Specific epithet) is derived from the Punjabi name of the species which means bitter [8]**.** While, in Hindi commonly as kutki whereas in Sanskrit kutka. Genome size 3452.34Mbp based on literature [9].

Endophytic association and metabolites production have been shown to hampered in this plant, these possibility for endophytic biosynthetic potential of metabolites and plant specific metabolites for substantial commodities [10]**.** Geospatial pattern of the species *Picrorhiza* have been explored in entire Himalaya region including Kumaon Himalayan range between 2,800 and 4,800 m altitude. Uttarakhand plant populations are scanty. Almora, Chamoli, Tehri, Pithoragarh and Uttarkashi as well certain selected fields are under cultivations of *Picrorhiza kurroa.* Different parts of plants such as rhizomes, roots, leaf, stem and seeds of *Picrorhiza* species contain about 132 constituents mostly from rhizomes which shows the presence of iridoids, cucurbitacins and phenolic glycosides. This plant is the rich source of picroside I and picroside II as a major bioactive compound, also contains pikuroside, phenol glycosides, veronicoside, cucurbitacin glycosides [11]. Various principles from *P. kurroa* are shown below. (Table 1)

Table 1: Various principles from *P. kurroa*

S. No	Bioactive Principles	Active constituents/compounds	Some cited structures	Some cited Mass	Some cited formulae	Literature
(i)	Iridoid glycoside	Picroside I	R1=OH, R2=OH, R3= 	492.1619	$C_{24}H_{28}O_{11}$	[12]
		Picroside II	R2=OH, R3=OH, R1= 	512.1859	$C_{23}H_{28}O_{13}$	[13]
		Picroside III	R1=OH, R2=OH, R3= 	538.1521	$C_{25}H_{30}O_{13}$	[14]
		Picroside IV	R1=OH, R2=OH, R3= 	508.1772	C24H28O12	[15]
		Picroside V	R1=OH, R2=OH, R3 			[16]
		6- Ferulocylcatalpol	R2=OH, R3=OH, R1= 	510.492	$C_{24}H_{30}O_{12}$	[17]

		Veronicoside	R2=OH, R3=OH, R1= 	814.699	$C_{35}H_{42}O_{22}$	[17]
		Minecoside	R2=OH, R3=OH, R1= 	538.502	$C_{25}H_{30}O_{13}$	
		Kutkoside	R1=OH, R3=OH, R2= 	512.1522	$C_{23}H_{28}O_{13}$	[18]
		Kutkin		496.465 g/mol	$C_{23}H_{28}O_{12}$	[19]
(ii)	Phenolic glycoside	Vanillic acid		168.0441	$C_8H_8O_4$	[19]
		Apocynin		166.0651	$C_9H_{10}O_3$	[20]
		Picein		298.291	$C_{14}H_{18}O_7$	[17]
		Androsin	 (14)	328.317	$C_{15}H_{20}O8$	[17]
(iii)	Cucurbitacin glycoside	25-Acetoxy-2-β-glucosyloxy-3,16,20-trihydroxy-9-methyl-18-norlanost-5,23-dien-22-one				[21]

Picrosides generally are monoterpenes (C10)/ terpene glycoside active molecules. Biosynthesis generally proceeds as usual through geranyl pyrophosphate (GPP). Some pathways are depicted shown in (Figure 3)

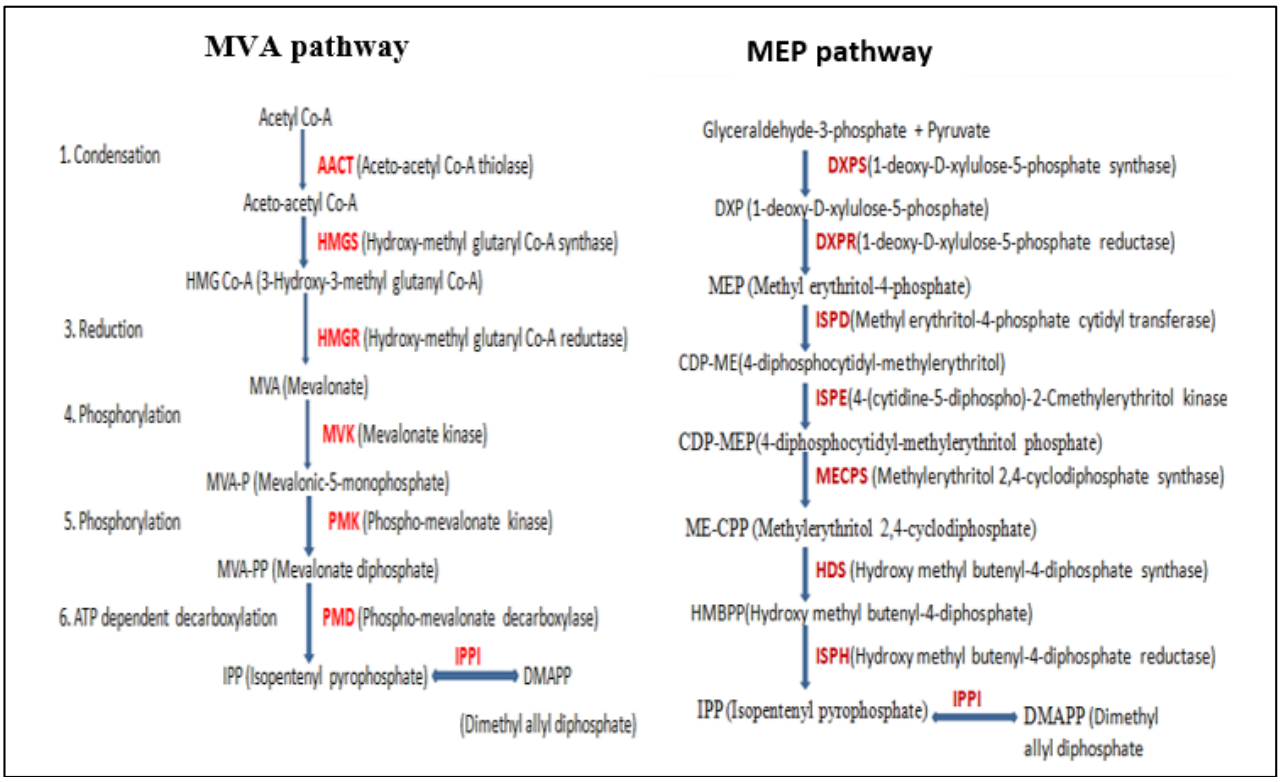


Fig 2: Various pathway depicted

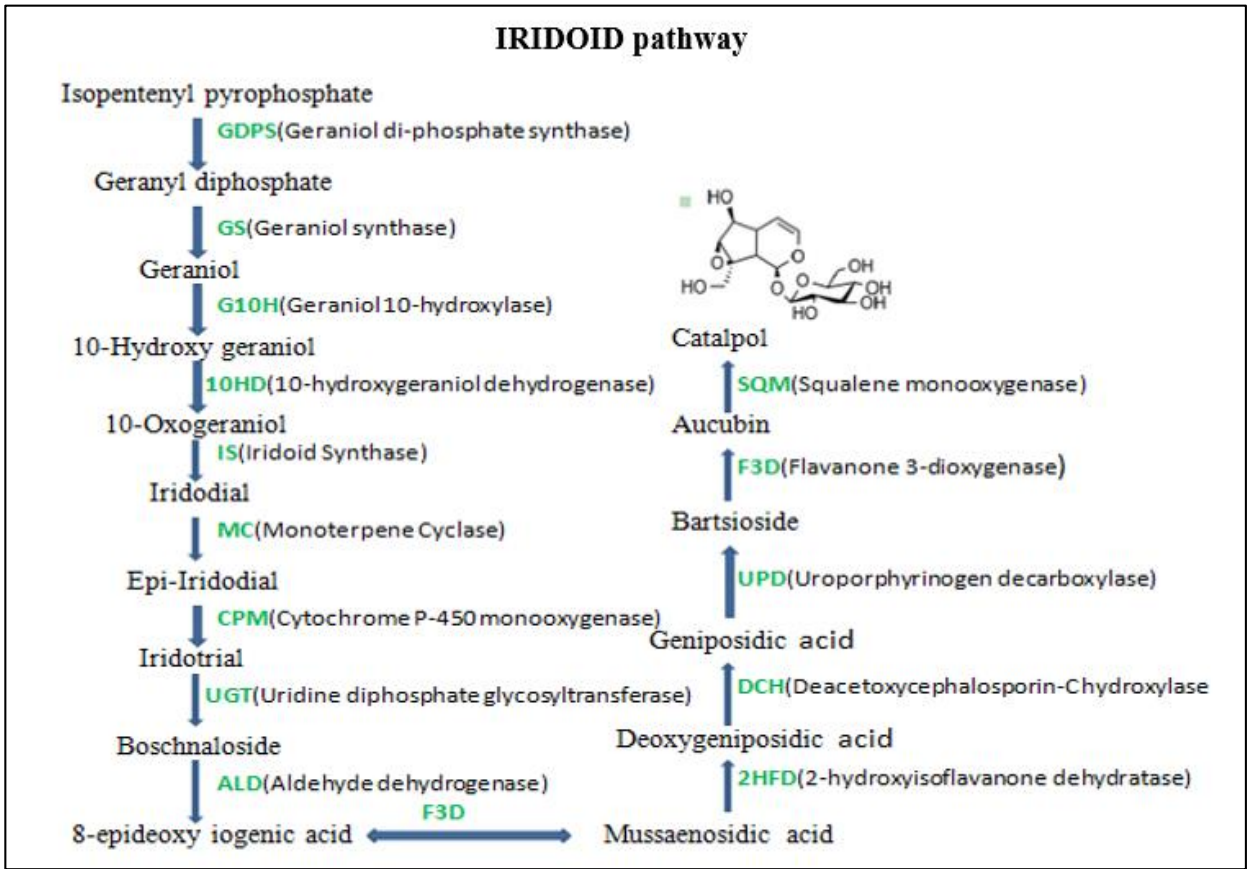


Fig 3: IRIDOID pathway

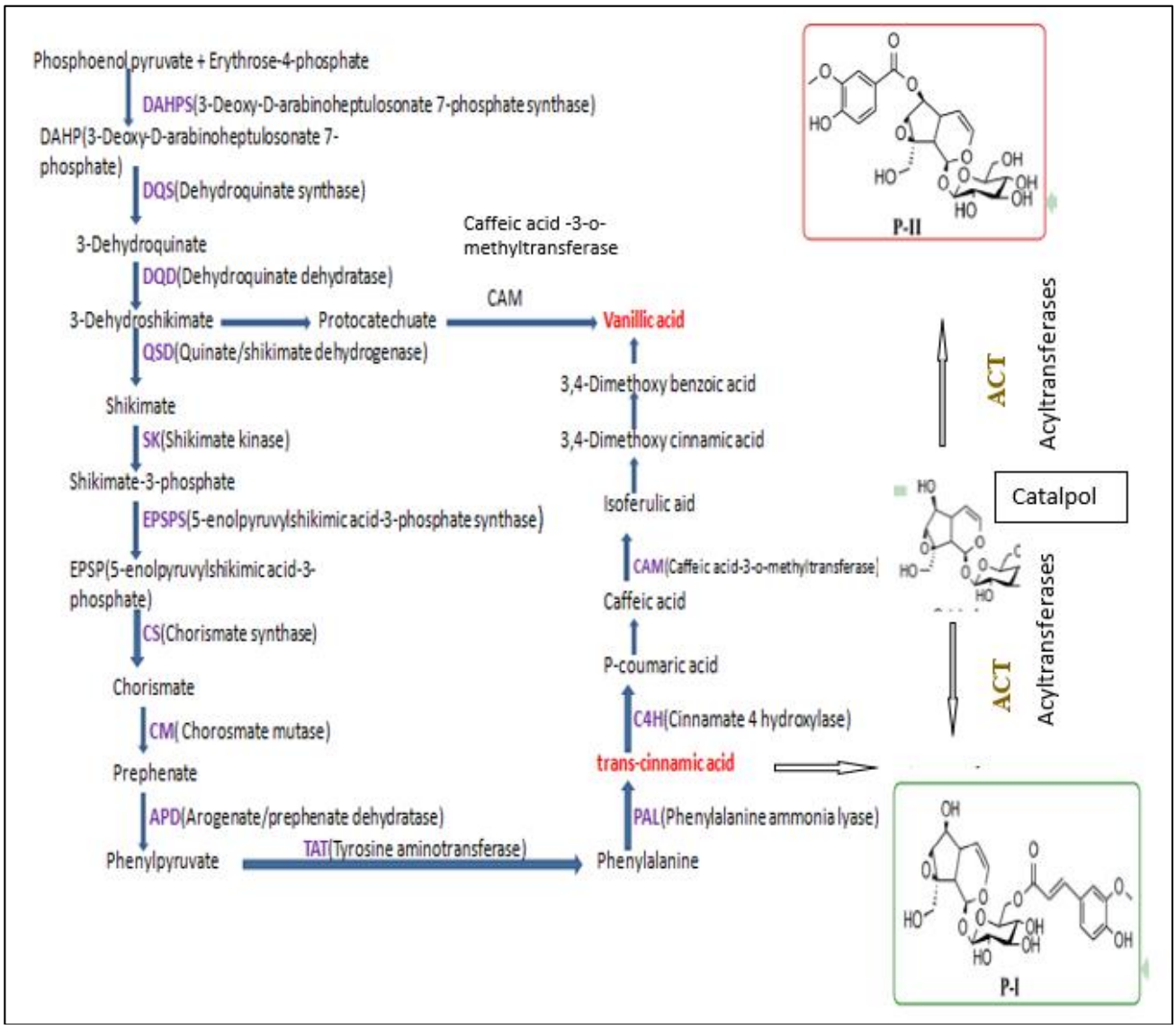


Fig 4

Cellular effects of active compounds/constituents

These are well defined for this particular plant as mentioned in different literature.

Increase in the level of blood serum glutamate pyruvate trans-aminase enzymes is one of the major markers for hepatocytes which leads to hepatic injury [22]**.** Substantial hepatoprotective action against carbon tetrachloride treated rats [23] and *Amanita* poisoning [24]and its reversal. The plant extract has shown improvement in intestinal adsorption also thus could be explored for nutraceutical potential [25]**.** Anti-inflammatory action reveals from the report of the inhibition of edema upto 29.8% [26]. Since anti-inflammatory and anti-analgesic effects are reported therefore the lead molecules may be designed with pharmacogenomics tools. Antioxidant properties has been established by lipid peroxidation [27]. The immune response of bio-polymeric fraction RLJ-NE-205 delivery system analyzed and reported induction of humoral and cellular immunity [28]. Antidiabetic activity of root extract of the plant in streptozotocin-nicotinamide stimulated type-2 diabetic rats had been also [29]. Certain leads for managing malignancy [30]**,** beside various saponins and flavonoids are responsible for Anti-asthmatic activity from this plant system based on model animal system [31]. Nephroprotective activity from this plant reported [32] and analgesic lead in albino mice model [33, 34] showed prohibitory actions of cardiac dysfunction induced by isoproterenol.

Propagation of *P. kurroa* mainly through roots and suckers. Its propagation through seeds is poor due to recalcitrance of seeds. Indiscriminate uprooting and killing for extraction of active ingredients without attention towards its replenishment and strategic cultivation of *P. kurroa* plants have led to depletion of its population in the nature.

Callusing in plant growth media supplemented differently. Shooting and rooting for regenerating efforts reported [35]**.** Silver nitrate effect and micronutrient effect were also observed upon total picroside content. Later reported callusing in the plant, further steps of much more refinement for *in vitro* regeneration [36, 37] reported in the same region for its growth. (table 2)

Table 2: Summary for *in vitro* procedures

S. No.	Explants Type	Nutrient medium (MS) Murashige skoog			Remark	References
		Callusing	Shooting	Rooting		
1	Shoot tips (from natural plant)	-	Kinetin (3–5 mg/L) + IAA (Indole-3-acetic acid) (1.0 mg/L) + Kn (3.0 mg/L)	NAA (Naphthaleneacetic acid) (1.0 mg/L)	Shoot initiation occurred in 28 days; root initiation in 7-9 days	[38]
2	Terminal and nodal cutting (from natural plant)	-	-	1.0µM NAA	Multiple shoots sprouted in 28 days and rooting was initiated in 18–20 days	[39]
3	Shoot tips			-	Callus and shoot maintenance	[40]
4	Leaf and nodal cutting from mature plant	2,4-D (0.5–2) +NAA (4) + Kin (1)	BA(6-Benzylaminopurine) (0.25 mg/L)	NAA (0.2)	Half – strength nitrogen was best for conversion of bud primordial in to shoots Callus induction within two weeks, shoot formation in six weeks Root initiation in 9 days	[41]
5	Cotyledonary node and shoot tips from in vitro grown seedlings	-	1.0 µM BAP	1.0 or 2.5 µM IBA	Plantlets and rooting	[42]
6	Nodal segments of natural plant	-	1.0 µM BAP	1.0 or 2.5 µM IBA)	Axillary shoots after 2 weeks and rooting after 11 days of transfer to PGR-free medium	[43]
7	Leaf discs, nodal segments and root segments	IBA (2mg/L) + Kin (3 mg/L) + table sugar (3%)		IBA (3) +table sugar (3%)	Low cost in production due to use of table sugar	[44]
8	Leaf discs, nodal segments and root segments were taken from in vitro grown plantlets	2,4Dichlorophenoxyacetic (2 mg/L) +IBA (0.5 mg/L) + 3% sucrose	BA (2 mg/L) +Kn (3 mg/L)	IBA (3 mg/L) + NAA (2 mg/L)	Root induction in 9 - 10 days	[45]
10	Nodal segments (from germinated seeds and mature plant)	2,4-D (0.25) + BA (0.25)	NAA (0.2 mg/L) MS + NAA (0.6)	NAA (0.4 mg/L)	Callus, direct and indirect organogenesis and rooting	[46]
11	Nodal segments, Leaf tissue of in vitro plantlets	Kin (2 mg/L) + IBA (0.5 mg/L)	Kin (2 mg/L) + IBA (0.5 mg/L)	IBA (1.0 mg/L)	Callus induction took place 20 days after inoculation from leaf explants	[47]
12	In vitro derived leaf disc, nodal segment, root	2,4-D (2 mg/L), IBA (0.5 mg/L),	(BA) (2 mg/L), KN (1.0 mg/L) and IBA (1.0 mg/L).	INA	Callus induction took place in 6–7 days, shoot primordia formation after 5 weeks	[48]
13	In vitro derived leaf, shoot tips, nodal segments (Micropropagation via synthetic seeds)	-		1 µM NAA	Rooting was initiated within 2 weeks	[49]
14	Leaf explants from *in vitro* shoot cultures	B5 + Kin (3mg/L) + IBA (1 mg/L)	B5 + Kin (3 mg/L) + IBA (1 mg/L)	B5 + Kin (3 mg/L) + IBA (1 mg/L) + activated charcoal (10)	High frequency (94%) multiple shoot bud regeneration occurred in 21–28 days which proliferated to shoots (10–12) shoots/explant) in 42–56 days. Shoots rooted in 21–28 days	[50]
15	leaves and stems	Leaf: TDZ(Thidiazuron) (0.5 mg /L) + IBA (0.3 mg/L) Stem: TDZ (0.5 mg/L) + IBA (0.5 mg/L)	Leaf: 1.0 mg L-1 BA + 0.75 mg L-1 KN Stem: 1.0 mg L-1 BA + 1.0 mg L-1 Kinetin	Leaf: NAA (0.4 mg/L) + 2-4, D (0.5 mg/L) +NAA (0.4 mg/L) Stem: 2-4, D (0.5 mg/L) + NAA (0.4 mg/L)	Callus, shoot induction and rooting	[51]
16	Shoot	-	BAP (1.0mg/L), Kn (0.5 mg/L) and GA3(1.0mg/L)	IBA (2.5 mg/L)	Multiple shoots and rooting	[52]

Figure 2 Upon elicitation, a series of metabolic reactions are systemically initiated in biological sphere to active differently [54-58] Physical effects like cold shock, UV effects enzymatic activities, however elicitation [59] is very complex cellular mechanism which had been supported by many researchers [60- 62]. One such cascades of events reported effluxes of K^+/ Cl^- and influxes of Ca_2^+/H^+ influxes [63-66] leading to metabolic pathways while growth of plants in different regimes some usual observation makes new aspect for biotechnological applications in each kind of plant [67]

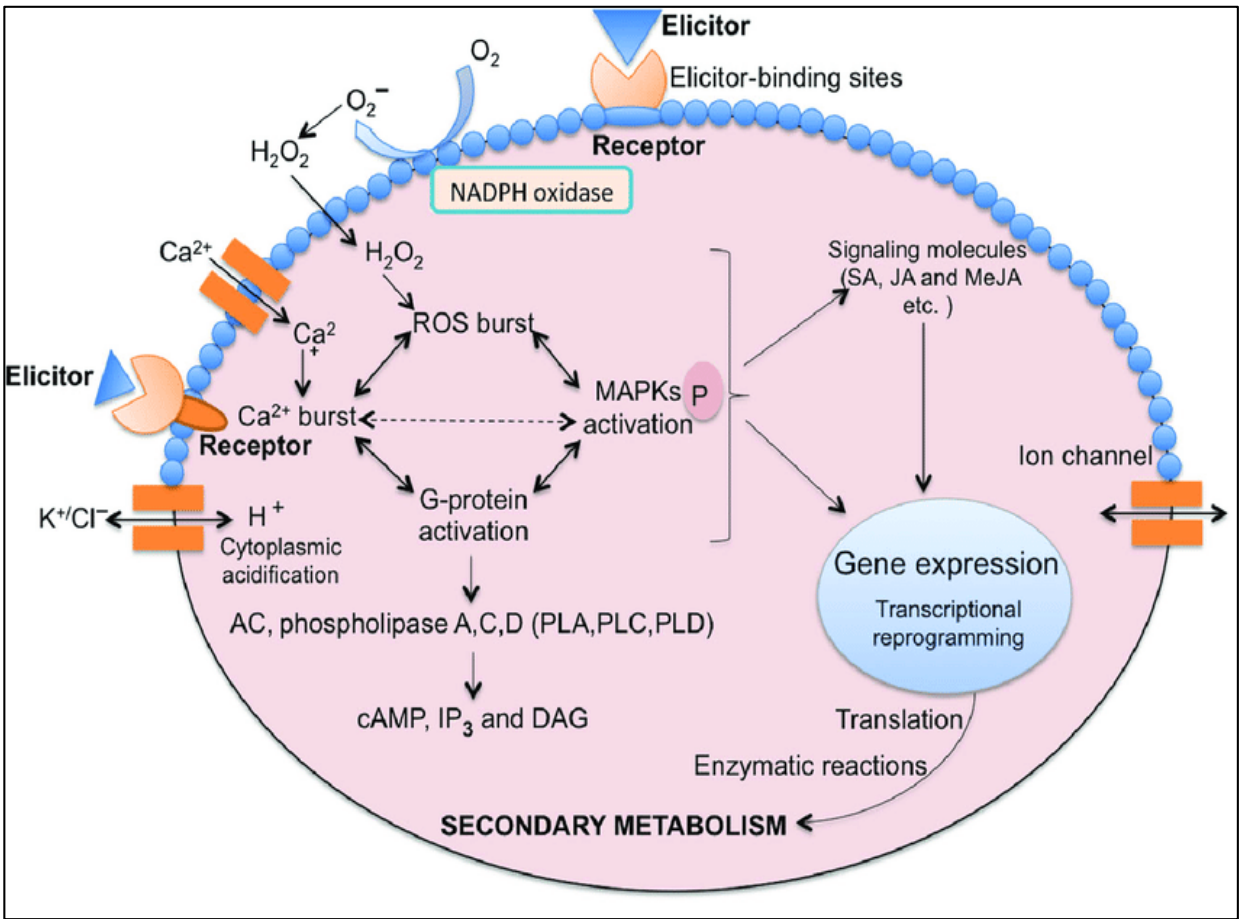


Fig 5: Signaling for secondary metabolites [53].

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