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Antifungal activity in *Brassica Juncea* by overexpression of bjnpr1

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Abstract
Brassica juneca also known as the mustard is a very useful oil seed crop which has a huge risk of loss of yields due to the biological factors due to which B. junecea have it's own antimicrobial activity to fight from these biological factor different types of mustard have different capacity to fight different microbial activity. B. juneca shows some best results in the variations among the different types of mustard which can also help in forming her4bal medicine. The extracts of B. juneca such as defesin, chitinase from the seed gave the anti-fungal activity which after tested gave variations for different types of Mustard. The tests were taken out on different microorganisms such as Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Candida albicans, Erwinia Carotovora, Proteus vulgaris Eterobacter cloacae, Shigella sp. and Streptococcus pyogensis. After the result we have different capacity of B. junecea for these microorganisms.

Keywords: *brassica juncea*, antimicrobial activity, phathogenic microbes, herbal medicine

Introduction
Plants are very important organism and are generally at a high risk of getting infected by the different microorganisms due to which they have evolved to create a defensive barrier towards the microorganism and to overcome different challenges (Roux *et al* 2014) [44]. Plants display performed defense as well as induce defense to overlay these challenges. Among both the defense induce defense is the most stronger defense and can prevent the plants for a high duration. Systematic acquired resistance (SAR) is the induce defense which give an better resistance to a variety of microorganism (Durant *et al* 2004). In plants the SAR response is accumulated by the infections and generate a response against that infection (Fu *et al* 2013) [20]. Many of the research have shown that the SAR response is dependent on the levels of the endogenous salicylic acid and the activation of a pathogen related (PR) gene. There are many PR proteins as glucanases, chitinases, thaumatins, and defenins have the antifungal activity and they have a major part in the resistance towards the disease. The extrensic work of SA is also been noticed in the stimulation of the SAR pathway (Durant *et al* 2004). Along with the SA a group of different proteins is also necessary for the stimulation of the SAR. From the different proteins the NPR1 protein id the main protein for the SAR pathway. The path to discover the SA receptor got to the discovery of the NPR1 protein (Cao *et al* 1994) [7]. Many of the research have said that the NPR1 is related to the SA singlanilng but its part in the process is largely unknown. After the attack of the pathogen on the plants they produce various phytoharmones and the composition, amount and the activation time of there harmones depend upon the species of the plant and the way of infection (De-Vos *et al* 2005) [15]. The SA pathway mainly work against the biotrophic microorganisms where other pathway like jasmonic acid/ethylene (JA/ET) pathways work against the necrotrophic pathogen (Glazebrrok *et al* 2005). Many of the elements that are present in SA/JA are found but the NPR1 among all the elements has the main role in the SA resistance by JA pathway (Spoel *et al* 2003) [46]. At the start is was found in Arabidopis, then afterwards different AtNRP1 were found in different crops. In the Arabidopisis NPR1 has more than one gene i.e. AtNPR1 and AtNPR2 an the main elements to perform the SAR (Cao *et al* 1997) [8]. On the other hand the AtNPR3 and AtNPR4 appose the SAR (Fu *et al* 2012) [21]. Many of the SAR theory was on the Arabidopsis NPR1. When the structure of the AtNPR1 was studied found that it contains ankyrin repeate, N-termional BTB/POZ broad-complex, Tramtrack, Bric a brac/poxvirus and zinc finger domain (Cao *et al* 1997) [8]. The NPR1 In its rest form is an oligomer form into cytosolic region. After the formation of SA the redox condition changes inside the cell which break the inactive oligomer into a active monomer and after is these monomer are trans located to the site where the TGA factors and the monomers attach to each other by the help of PR gene (Mou *et al* 2003) [37]. The observations have told that the NPR1 is shown after the attack of the microorganism or the plant gets the resistance by the SAR the other biological factors. As observed that the plants that have the NPR1 or that have introduced NpR1 artificially are best at resisting the microbes (Glazebrook *et al* 1997). As its also observed that the NPR1 has a main role in the signaling and the resistance that's why it is also knows as the main inducer of the resistance from SA (Spoel *et al* 2003) [46]. To know the mechanism of defense by the NPR1 many studies have be done on the artificial system as well as the real plant system. NPR1 regulate the translation of the PR gene and SAR which is done by the SA. When the NPR1 is over expressed is cause the increase in the transcripts of the PR1, PR2 and PR5 (the anti-fungal genes) which are mainly have the antifungal activity. According to the different studies the capacity to resists the large variety pathogen of the PR gene is shown. The PR gene is mainly activated by the transportation of the NPR1 by the redox of the nucleus (Cao *et al* 1998) [9]. As it has been seen in the Arabidopsis that

when the NPR1 is expressed at high levels id resistance towards the microorganism is highly increased. In the carrot also when the transgene is added the plant showed the resistance towards variety of the microorganisms like biotroph and the necrotrophic fungal pathogen (Wally *et al* 2009) [50]. It is also been given that when the transgene is add in the cotton it resist the fungus as well as the nematodes (Parkhi *et al* 2010) [40]. All these results we have give that the NPR1 id the main and the important gene for the fight against the microorganisms and is the key element for the transgenic crops that's resist the pathogen. Brassica juncea is basically the main oil seed crop which completes the 27% of the need of the vegetable oil of the country (Giri *et al* 2013) [22]. But the fungal infection mad the loss by decreasing the yields (Chandrashekar *et al* 2015) [10]. As yield B.juncea have to face many infectious organisms as Alternaria brassicae, Albugo candida, Sclerotinia, Sclerotiorun and many more. As in normal B. juncea is not able to resist the pathogens and it does not have the genes which form the enzymes against the pathogens. We can use different material to resist these pathogens but those materials are high in rates and its effectiveness can also have a impact due to weather. So the gene like NPR1 that is able to influence different genes that can help in the defence from the pathogens and can form a defence from a large variety of pathogen (Bal etal 2014) [4]

Articulation example of BjNPR1 under Harmonal medicines and Fungal Infections

We have took different B. juncea to check the fungal growth in them. These plants were sprayed with different solutions (2 mM SA, 100 µM JA and 50 µM abscisic acid) and the controlled plants were splayed with the sterile water which was mixed in different solvents also used for the development of the harmones in the same amount. Afterwards the leaf of the plants were took after different hours (0,2,4,8,12,24,48,72) of the treatment to check the infections in different levels, One of A. brassicae strain was taken and grown in 22°C for the upcoming 15 days(Thakur *et al* 1985) [49] and the spores thet were formed were used to form the inoculum then the B. juncea(40 days old) was infected by the inoculum. A pure culture of E. cruciferarum which was isolated in the lab was user to infect the B. juncea (40 days old) for the powdery mildwe infection. (Aravind *et al* 1999) [2] These infected plants were stored in the growth chamber at a relative humidity of 100% and at a temperature of 22°C in which the controlled plants that were incubated seperately so that they not get contaminated buy the infected plants Now the from the plants were taken at different time and were frozen and put at the temperature of -80°C

RNA Isolation and Reverse Transcription Quantitative PCR

To dissect the statement of BjNPR1 after hormonal medicines what's more, contagious disease, invert record quantitative PCR (RT-qPCR) was performed utilizing BjNPR1 quality explicit preliminaries. All out RNA was disengaged from 100 mg of leaf test gathered from treated and control plants utilizing Ambion RNA detachment pack as depicted by maker's convention (Life Advancements). (Bari 2009) [5] Reciprocal DNA (cDNA) was blended from 2 mg of sanitized all out RNA by invert transcriptase in 20 ml response volume containing oligo(dT) 18 groundworks, 10 mM deoxynucleotide (dNTPS) and water following the producer's directions (Invitrogen, Canada). RT-qPCR response blend contains 2 µl of cDNA, 5 µl of SYBR green continuous PCR ace blend (Takara, Japan) and 0.5 µl (10 pmol) of every preliminary (BjNPR1). The RT-qPCR thermocycling program was following: 95ºC for 5 min, trailed by 40 cycles at 94ºC for 30 s, at 60ºC for 30 s, and at 72ºC for 30 s.

All preliminaries utilized in this examination were planned by Oligoanalyzerprogramming (Table 1). The alpha tubulin quality (GenBank increase no-NM_100360.) was utilized as reference quality for standardization of articulation esteems (Makandar *et al* 2006) [34]. The general articulation levels of BjNPR1 were measured by different technique (Livak *et al* 2001) [33] All responses were directed with three natural repeats. Overlap changes with p-values under 0.05 were thought of huge

Binary Vector Construction and Agrobacterium Transformation

The full length cDNA of BjNPR1 was cloned in sense course into pBI121 at Sma1 and Sac1 site downstream of constitutive advertiser 35S CaMV (cauliflower mosaic infection). (Kinkema *et al* 2000) [27] The right direction of BjNPR1 piece in the recombinant plasmid was additionally recognized by PCR and sequencing. The subsequent recombinant double vector pBI121-BjNPR1 was assembled into Agrobacterium tumefaciens EHA105 by freeze-defrosting strategy (Holsters *et al* 1987).

Brassica juncea Transformation

Brassica juncea cv. Varuna seeds were sprouted on half quality Murashige and Skoog (MS) medium in Magenta boxes (Red vessel Corp., United States) at 24 ± 2ºC under cool white rich light (90–150 mmol photons/m2s) in a 16/8 h (light/dim) photoperiod (Murashige *et al* 1962) [38]. BjNPR1 transgenic plants were created through Agrobacterium intervened co-development strategy as per convention with a few alterations (Sharma *et al* 2009) [45]. Seeds acquired from T0 changed B. juncea plants were essential screened on kanamycin determination medium and afterward moved into pots for further investigation.

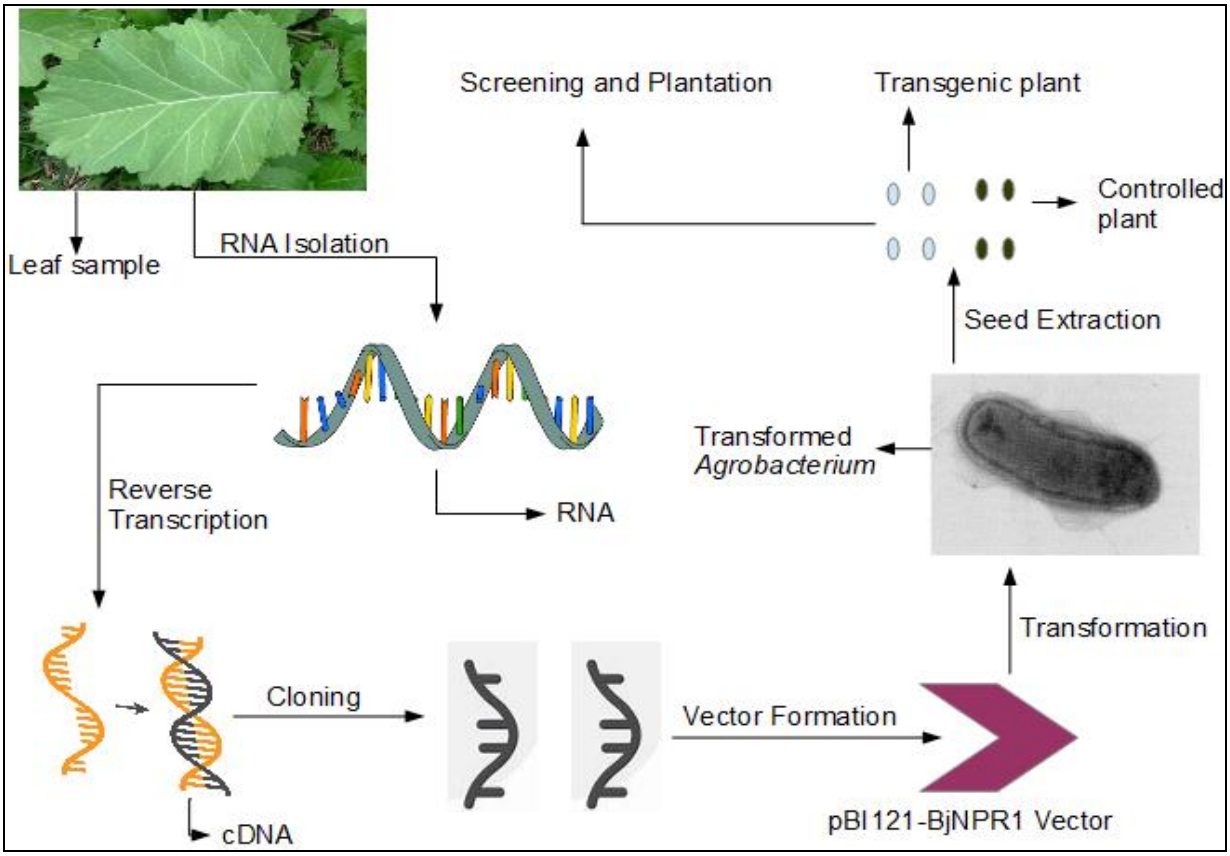

Fig 1

Molecular Screening of BjNPR1 Transformants

Genomic DNA was disengaged from putative BjNPR1 changed what's more, non-changed plants following CTAB technique. For atomic screening of BjNPR1 transgenic plants PCR and Southern smear examination was utilized. PCR identification of BjNPR1 transgene was completed utilizing 35S advertiser (forward) and BjNPR1 (turn around) preliminaries (Rochon *et al* 2006) [43]. Southern smudge investigation was performed to distinguish the transgene addition and the duplicate number utilizing Burrow High Prime DNA Labeling and Detection Starter Kit I (Roche Applied Science, Mannheim, Germany). For combination, a 600 bp section of 35S advertiser (forward preliminary) and NPR1 (turn around preliminary) were utilized as a test in BjNPR1 transgenics. To distinguish duplicate number of BjNPR1 in transgenic plants, NPTII test (500 bp) was utilized. (Durrant *et al* 2004) [17]

Expression Analysis of NPR1 and PR Genes in BjNPR1 Transgenics

RT-qPCR was performed to screen the record levels of the BjNPR1, PR1, PR2 (b 1-3 glucanse), PR3 (chitinase), PR5 (thaumatin), PR12 (defensin), and PR13 (thionin) in the leaf tissue of the transgenic and non-transgenic plants. RNA segregation, cDNA union and RT-qPCR tests were executed as portrayed in the above area. (Delnay *et al* 1995)

Phenotypic Characterization of BjNPR1 Transgenic Plants

Distinctive agronomic attributes in particular, size and state of leaf, siliques, blossoms, number of units, number of seeds also plant tallness were explored for any phenotypic irregularities between BjNPR1 transgenic and wild-type B. juncea plants. (Cao *et al* 1994) [7]

Necrotrophic and Biotrophic Resistance Assay

BjNPR1 transgenic lines were assessed for infection protection from both necrotrophic and biotrophic parasitic microbes. Alternaria contamination in BjNPR1 transgenic lines and non-changed plants

Were completed as portrayed in our past work (Ali *et al* 2017) [1]. For infection scoring, three segments of fractional obstruction. counting sore appearance, number of injuries per leaf, sore measurement (mm) and level of illness leaf region (%DLA) were estimated and analyzed between BjNPR1 transgenic lines and control plants after immunization. Illness seriousness was determined following 15 days immunization (DAI), with a 10-point infection rating scale.(De-vos *et al* 2005) [15] For, E. cruciferarum disease, 40 days old solid BjNPR1 transgenic and wild-type B. juncea plants were contaminated as portrayed in above segment. The immunized plants were kept up at 22ºC with 100% RH in an immunization chamber. Infection aggregate was analyzed at 7 days after fine mold immunization. For sickness scoring, various boundaries were utilized for example, province appearance, number of settlements or spots, illness record, rate infection leaf territory (%DLA) between BjNPR1 transgenic lines and control plants. Sickness list including six grades: 0, 1, 3, 5, 7, and 9, which compare, individually, to sickness frequency levels of 0, <5%, 6–10%, 11–20%, 21–40%, furthermore, >41%). The analyses were done in three organic repeats. (Glazebrook *et al* 2005) [23]

Trypan Blue Staning and Microscopy

Trypan blue recoloring was utilized for watching dead cells and parasitic biomass in charge and BjNPR1 transgenic plants. Quickly, control and transgenic tainted leaves were recolored with trypan blue recoloring arrangement

[containing 40 mg of trypan blue, 10 mL lactic corrosive (85% w:w), 10 mL phenol (pH 7.5–8.0), 10 mL glycerol (>99%) and 10 mL of refined water] for 30 min at room temperature. The examples were washed with sterile water to eliminate the overabundance stain and afterward drenched in 70% ethanol arrangement short-term to eliminate the chlorophyll. The arrangement was then taken out and tissue tests were inundated in 60% glycerol and captured. (Dutt *et al* 2015) [18] The contagious biomass and cell demise was imagined under light magnifying lens (10 xs, Nikon).

Statistical Analysis

For all tests, three natural duplicates were utilized and each rehashed multiple times. An understudy's t-test was completed to decide critical contrasts in BjNPR1 quality articulation in charge and regarded tests also transgenic and nontransgenic plants. The contrasts between two gatherings of information for correlations in all the investigations were assessed as factually huge (*$p < 0.05$) or incredibly critical (**$p < 0.01$). (Fu *et al* 2012) [21]

Isolation and In Silico Analysis of BjNPR1

The full-length cDNA of BjNPR1 quality was disconnected from SA treated B. juncea library, and submitted to the Genbank with increase number DQ359129. In silico investigation of BjNPR1 cDNA uncovered that it is included 1781 bp with an open perusing edge of 1857 bp, encoding a protein of 593 amino acids with a sub-atomic mass of 65.77 kDa, and a hypothetical PI of 5.25. Phylogenetic investigation demonstrated that BjNPR1 is extremely close homolog to NPR1 of B. napus, B. oleracea, B. rapa, and A. thaliana, separately, however was generally veered from NPR1 of Poaceae family. (Spoel *et al* 2003) [46] Arrangement of found amino corrosive grouping of BjNPR1 (increase no. ABC94642) uncovered 92% character with BnNPR1 (increase no. XP013725724), 78% with BolNPR1 (increase no. XP013605797), 78% with BrNPR1 (increase no. XP009109186), and 66% with AtNPR1 (increase no. ABR46023). To research the commonplace area structure of BjNPR1, its protein succession were dissected utilizing Pfam programming which uncovered the anticipated BTB, ANK preserved area (Wally *et al* 2009) [50]

Articulation Analysis of BjNPR1 in B. juncea after Hormonal Treatments and Fungal Infection

The articulation example of BjNPR1 was researched under different hormonal burdens (SA, JA, and ABA) just as vaccination with A. brassicae and E. cruciferarum through constant PCR. Upon SA treatment, record levels of BjNPR1 began to increment at 2 h (2.9-crease) and arrived at a top at 12 h with a sharp decay at later time focuses. Then again, no critical acceptance of BjNPR1 was seen in JAtreated plants and stays as before as control (Campbell *et al* 2010) [40]. Treatment of B. juncea plants with ABA diminishes the record levels of BjNPR1 at 2 h (0.65-overlap) and stayed low until 72 h time span. It has been all around recorded that NPR1 assumes basic part in infection obstruction in plants. To additional examination the protective function of BjNPR1, we vaccinated B. juncea plants with both necrotrophic (A. brassicae) and biotrophic (E. cruciferarum) contagious microbes. After Alternaria vaccination, the outflow of BjNPR1 was somewhat expanded at 4 h, arriving at most extreme at 12 h (2.7-overlay) of post immunization (Chanderashekar *et al* 2015). Then again, immunization of B. juncea plants with E. cruciferarum drove higher up-guideline of BjNPR1, and the most elevated articulation levels were seen after 72 h (6.11- crease) to 96 h (6.33-overlay) contrasted with contro. Consequently, these outcomes recommend that BjNPR1 is initiated by contagious microorganisms and might assume significant part in B. juncea illness opposition. (Thakur *et al* 1995)

Table 1

Plants Treated With	Transcripts levels of BjNPR1
Salycilic Acid	Increase at 2 h (2.9-fold) and reached a peak at 12 h with a sharp decline at later time points
Jasmonic Acid	No induction of BjNPR1 as same as control
Abscisic acid	Decrease in a transcript level of BjNPR1 at 2 hour and remained low after 12 hour as well
Inoculating *Alterneria*	Level of BjNPR1 slowly increase at 4 h then reach at maximum at 12h
Inoculating *E. cruciferarum*	Highest Expression level of BjNPR1 was at 72h to 96h

Development and Molecular Analysis of Transgenic B. *juncea L*ines Overexpressing BjNPR1

So as to additional affirm the guarded function of the BjNPR1, transgenic B. juncea lines with constitutive articulation of BjNPR1 were created through tissue culture strategy to improve the resistance (Supplementary Figures S1A–J) (Bal etal 2014) [4]. For this reason, full length compact discs of BjNPR1 was cloned into pBI121 parallel build heavily influenced by CaMV 35S constitutive advertiser. Further, BjNPR1 transgenic plants were created utilizing Agrobacterium hypocotyls co-development strategy (Valuable Figure S1). In the current investigation, generally speaking change adequacy was discovered to be 2.7% utilizing co-development strategy (Supplementary Table S1). A sum of 10 transgenic lines were gotten through kanamycin screening, and T-DNA mixes were affirmed by PCR enhancement. Overexpression of BjNPR1 was inspected in transgenic lines by q-RT PCR (Holsters *et al* 1978) [26]. The mRNA levels of the BjNPR1 quality fluctuated enormously in various lines, for example, lines 2, 5, 7, and 8 appeared high collection of BjNPR1, while remaining lines (1, 3, 4, 6, 9, and 10) uncovered moderately low articulation levels of BjNPR1. To additionally explore the reconciliation and duplicate number of BjPR1 transgene in chose exceptionally articulation lines (2, 5, 7, and 8), Southern smearing was performed utilizing 35S-NPR1 also, NPTII tests, and all the four lines indicated 35S-BjNPR1 combination, individually. Moreover, single duplicate addition was seen in T-DNA lines 2 and 5, where as two duplicates were found in lines 7 and 8. At last, single duplicate number and high articulation lines of BjNPR1 transgenic (lines 2 and 5) were chosen for infection screening. (Rawat *et al* 2017) [1]

BjNPR1 Transgenic Plant Modulates the Expression of SA and JA Signature (PR) Genes

Microorganism related qualities are not just known as sub-atomic marks of the SA and MeJA flagging pathways yet additionally broadly utilized as demonstrative markers in microorganism obstruction examines. For the most part, overexpression of NPR1 qualities has been appeared to

improve the resistant reaction (purported preparing) through the enactment of SAR marker or PR qualities. Here, we inspected regardless of whether B (Brader *et al* 2014). juncea plants overexpressing BjNPR1 quality will lead to acceptance of PR qualities. For this, we contemplated the articulation of SA and JA flagging analytic qualities (PR1, PR2, PR3, PR5, PR12, and PR13) in chose profoundly communicated BjNPR1 transgenic lines and non-transgenic plants under non-focused on conditions. The articulation levels of SA marker qualities PR1, PR2, and PR5 in BjNPR1 transgenic lines (L2 and L5) was discovered to be relatively higher than in charge (non-transgenic) plants (Dong *et al* 2004) [16, 17]. Then again, low articulation levels of JA signature qualities (PR3, PR12, and PR13) were seen in BjNPR1 transgenic lines when contrasted with SA signature qualities however were higher than in charge (non-transgenic) plants. These outcomes demonstrated that the constitutive articulation of BjNPR1 was related with the quicker and more grounded actuation of PR qualities which could upgrade illness opposition in B. juncea to numerous microorganisms. (Yuan *et al* 2007) [51]

Phenotypic Analysis of BjNPR1 Transgenic Plants

In this examination, relative investigations on phenotypic variations from the norm in BjNPR1 transgenic plants were efficiently assessed. Our results uncovered that all the examined agronomic attributes in particular, shape and size of leaves, siliques, bloom morphology, seed shape, number of cases, number of seeds and plants stature in BjNPR1 transgenic plants were comparative with that of wild-type plants (Després *et al* 2003) [14]. Subsequently, these outcomes gives the proof that BjNPR1 transgenic lines didn't show any phenotypic anomalies as was seen in other harvest plants subsequent to overexpressing AtNPR1. Out and out, this information demonstrated that BjNPR1 transgenic plants demonstrated ordinary development and improvement. (Bheker *et al* 2015)

Overexpression of BjNPR1 in B. *juncea* Transgenic Plant Confers Partial Disease Resistance to Necrotrophic Fungal Pathogen

To investigate the function of BjNPR1 in sickness opposition, we assessed obstruction level in BjNPR1 transgenic plants against necrotrophic (A. brassicae) parasitic microorganism, which is the most genuine microbe of B. juncea. We chose two transgenic lines (L2and L5) for this investigation dependent on articulation levels of BjNPR1 transgene. For Alternaria disease, BjNPR1 transgenic and control plants were contaminated and infection scoring was surveyed at distinctive time spans (Zhang *et al* 2015). After immunization, little necrotic injuries started following 3 days in non-transgenic plants and size of the necrotic injuries expanded essentially after infection movement. Interestingly, BjNPR1 transgenic plants additionally indicated injuries however the injury size or distance across was relatively lower than nontransgenics. Besides, our outcomes uncovered that infection seriousness was high in non-transgenic plants, and covering roughly 30% of the absolute leaf territory than the BjNPR1 transgenic lines at fifteenth dpi (Endah *et al* 2008) [19]
. The infection obstruction was evaluated by estimating the normal injury width in the Alternaria tainted leaves for both BjNPR1 transgenic and non-changed plants, and sore width was half diminished in the previous contrasted with non-transgenics. We additionally saw that expanded number of injuries spread on distal or on the other hand non-contaminated leaves in non-changed plants than BjNPR1 transgenic plants after Alternaria disease (Mazumder *et al* 2013) [36]. Based on illness file (0–10 scale), most elevated infection occurrence (3–4) was found in charge plants, while illness rate 1–2 was seen in BjNPR1 transgenic lines following contamination. Furthermore, we additionally screen the cell demise and parasitic biomass in transgenic and wild-type plants after Alternaria contamination utilizing trypan blue recoloring (Chern *et al* 2005) [11]. In light of the infinitesimal assessments, the contamination expanded the quantity of dead cells with bigger also, extending cell passing regions saw past the immunization site in non-transgenic plants contrasted with transgenic lines. Additionally, the parasitic biomass and spore load after sixth, twelfth, and fifteenth of Alternaria contamination were essentially low in transgenic lines than that of non-transgenic B. juncea plants. In this manner, our outcomes uncovered that BjNPR1 transgenic plants show halfway protection from Alternaria leaf scourge as there was delay in injury appearance, size, and spread of contamination in contrast with non-transgenic plants. (Canlas *et al* 2005) [11]

BjNPR1 Plants Showed Improved Resistance agains Poowdery Mildew.

In the current examination, we additionally inspected the opposition level of the BjNPR1 transgenic lines against fine buildup illness, another significant contagious sickness of B. juncea caused by biotrophic microorganism (E. cruciferarum) which is completely diverse in method of disease style and flagging pathways from A. brassicae (Kieffer *et al* 2011) [30]. To survey the obstruction level of BjNPR1 transgenic lines against fine mold, plants were contaminated what's more, sickness scoring was accomplished for 1–3 weeks. In nontransgenic plants, higher number of E. cruciferarum provinces was seen than transgenic lines on seventh, twelfth, and seventeenth day after contamination. In view of state check, there was roughly half decrease in recently shaped states between transgenic lines and non-transgenic plants. At seventeenth day of contamination, transgenic plants indicated fine buildup contamination with an illness size of 3–4 (30–40%), while as non-changed leaves (wild sort) uncovered 7–8 (70–80%) of sickness frequency, individually (Lehenanf *et al* 2011). Furthermore, E. cruciferarum intervened cell demise was analyzed in BjNPR1 transgenic and wild-type plants at various time focuses utilizing trypan blue recoloring and light microscopy. Based on infinitesimal perceptions, more cell demise was watched in charge than that of transgenic plants. To further examine the function of BjNPR1 in improving fine mold sickness obstruction, the development or contagious biomass of E. cruciferarum in BjNPR1 transgenic lines with wild-type plants was analyzed utilizing light microscopy. Contagious biomass of E. cruciferarum than wild-type plants at seventh, twelfth, and seventeenth dpi. Notwithstanding, overexpression of BjNPR1 couldn't restrain the development of E. cruciferarum totally, along these lines giving just incomplete protection from fine buildup illness. Likewise, more number of leaves and cases were tainted in non-transgenic plants when contrasted with transgenic lines. These results show that BjNPR1 transgenic plants displayed halfway protection from fine buildup contamination, which was adequate to defer the spread of

contamination in non-tainted leaves or different parts. (Blanco *et al* 2009) [6]

Discussion

ID and understanding the part of guard administrative qualities is important to create illness safe transgenic crops in horticultural framework. Control of administrative qualities has numerous helpful jobs, for example, actuation of various protection qualities or pyramids which gives powerful and dependable security contrasted and a solitary quality methodology. Subsequently, in the present study BjNPR1 an administrative quality was detached and portrayed, furthermore, phylogenetic investigation of the anticipated BjNPR1 protein with other known NPR1-like successions uncovered that they are assembled into unmistakable clades. Nonetheless, BjNPR1 fall inside a similar clade as other Brassica family NPR1 proteins. In view of auxiliary investigation, BjNPR1 protein contains areas, for example, an ankyrin rehash area and a BTB/POZ space, which are exceptionally preserved in all NPR1 proteins (Cao *et al* 1997) [8]. These spaces are basic segments of NPR1 and give capacities identifying with NPR1-dependant co-enactment of TGA record elements and protein–protein authoritative (Rochon *et al* 2006) [43]. A period course articulation examination of BjNPR1 after protection hormonal medicines also, parasitic contaminations were completed, lastly BjNPR1 was overexpressed in B. juncea to display infection protection from Alternaria scourge and fine mold. Phytohormones, including SA, MeJA, ET, and ABA play an fundamental function in the guideline of plant resistant reactions to microbial microorganisms. Be that as it may, each sign atom or microbe has its particular component (Kunkel *et al* 2002) [29]. It is very much recorded that SA flagging pathway is engaged with the enlistment of SAR while as JA/ET are engaged with the initiation of instigated fundamental obstruction. NPR1 (an administrative protein) isn't just a bonafide receptor of SA yet in addition a positive controller of SAR, and assumes a crucial part in SA/JA flagging crosstalk (Li *et al* 2008). Freak npr1-1 plants are undermined in SAR as well as in basal obstruction against numerous kinds of microorganisms that are touchy to SA subordinate protections (Dong *et al* 2004) [16, 17]. Past examinations have uncovered that NPR1 assumes a focal function in the incited guard flagging organization that is constrained by SA, JA, and ET (Pieterse *et al* 2004) [41]. There is plenty of studies on safeguard flagging falls, however the greater part of them have been conveyed out in model plants. Along these lines, revealing the part of SA/JA ace controller (BjPNR1) in B. juncea will give novel experiences at sub-atomic level. Beforehand, the articulation energy of NPR1 or on the other hand its homologs was discovered to be expanded essentially after exogenous use of SA that prompts the enactment of SAR (Yuan *et al* 2007) [51]. Exogenous use of SA expands NPR1 record collection as well as changes its protein design in the core, essentially through posttranslational changes (Tada *et al* 2008) [48]. NPR1 and TGA1 are essential redox-controlled controllers of SAR in plants. By and large, NPR1 is found as an oligomer inside the cytoplasm of uninduced cells and changes in SA fixation lead to a changed redox climate inside the cell, driving the atomic limitation of NPR1 in its monomeric structure (Mou *et al* 2003) [37]. NPR1 monomers connect with the diminished type of TGA1, which focuses on the initiation succession 1 (as-1) component of the advertiser area of guard proteins (Després *et al* 2003) [14]. In expansion, SA-intervened redox adjustment likewise plays a significant function in the SA-interceded weakening of the JA flagging pathway (Koornneef *et al* 2008) [28]. In this examination, we found that SA expanded record levels of BjNPR1 in B. juncea, reliable with results saw in various yield plants (Zhang *et al* 2008) [52]. Then again, exogenous treatment with JA didn't adjust the declaration of BjNPR1, comparable results were likewise seen in avocado plants (Backer *et al* 2015) [3]. Notwithstanding, conflicting outcomes were found in rice and banana is by all accounts have explicit associations (Endah *et al* 2008) [19]. By and large, ABA not just assumes a focal part in abiotic stress signal transduction, yet in addition has been known to have positive or negative effect on plant resistant framework (de Torres *et al* 2007) [13]. Past reports have appeared that ABA advances NPR1 debasement in Arabidopsis. In this study, exogenous use of ABA diminishes the articulation of BjNPR1 when contrasted with mock treated plants. Numerous reports have demonstrated that ABA shows up upstream of NPR1 what's more, smothers the declaration of both NPR1-subordinate and autonomous flagging marks. Our outcomes further give the proof that ABA adversely directs NPR1, positive controller of SAR pathway in B. juncea. Through and through, our outcomes uncovered that NPR1 is particularly controlled by protection triggers, and furthermore affirms that BjNPR1 is probably going to be subject to SA flagging which was reliable with the arrangement examination information that BjNPR1 contained an atomic limitation signal (NLS1) that was basic for SA-interceded articulation of PR qualities. There is developing assemblage of confirmations that SA flagging triggers protection from biotrophic microorganisms, though a JA/ET pathway prompts protection from necrotrophic microbes (Glazebrook *et al* 2005) [23]. In the current examination, BjNPR1 was reasonably prompted by necrotrophic microorganism (A. brassicae) and the articulation is by all accounts JA free as it was not incited during JA treatment. (Mazumder *et al* 2013) [36] like wise revealed that A. brassicicola, a necrotrophic microorganism builds SA collection in B. juncea during beginning phases of infection advancement which may stifle the JA pathway for fruitful contamination. In our past investigation, we have additionally watched the enlistment of SA marker quality PR1 after A. brassicae disease (information not appeared). These outcomes propose that there is a hormonal crosstalk in B. juncea during Alternaria contamination which could trigger the outflow of NPR1 or SA subordinate qualities. As expected, record levels of BjNPR1 were essentially expanded during E. cruciferarum disease when contrasted with uninfected plants, like that saw by (Dai *et al* 2016) [12]. Besides, the articulation levels of BjNPR1 in fine mold tainted plants were generally higher than that of Alternaria tainted plants since microbes causing fine buildup illness are known as severe biotrophic microorganisms which depend on SA pathway (Oliver *et al* 2004) [39]. Past investigations have uncovered that Arabidopsis NPR1 at the point when changed into various yield plants indicated upgraded infection opposition which make NPR1 a promising and potential up-and-comer quality for creating infection safe transgenic plants. In this investigation, B. juncea transgenic plants were created by overexpressing BjNPR1 utilizing 35S advertiser through Agrobacterium intervened plant change.

It was before detailed that overexpression of AtNPR1 or its homolog OsNH1 in rice transgenic lines albeit improved protection from microbes however, indicated numerous hindering impacts, for example, chlorotic injuries, touchy to light and created higher measure of responsive oxygen species which drives cell passing(Chern *et al* 2005) [11]. Notwithstanding, in our investigation BjNPR1 transgenic plants displayed ordinary aggregates and didn't indicated any variations from the norm, and comparable discoveries were likewise seen in wheat, tobacco, and apple NPR1 transgenic lines. We next tended to regardless of whether BjNPR1 transgenic lines could enact SA or JA guard pathways by expanding the collection of guard marker qualities (PR qualities). The same number of studies have uncovered that transgenic plants overexpressing AtNPR1 initiates PR quality articulation in tomato, grape, tobacco, and rice (Le Henanff *et al* 2011) [30]. Nonetheless, opposing outcomes were additionally found in carrot plants where NPR1 overexpression lines didn't expand the record levels of PR qualities under ordinary conditions. In present investigation, overexpression of BjNPR1 fundamentally expands the record levels of SA subordinate PR qualities like BjPR1, BjPR2, and BjPR5. In any case, low acceptance of JA signature (PR) qualities was seen in BjNPR1 transgenic plants. NPR1 has been exhibited to be a significant transducer of the SA signal in the SA-intervened enactment of PR quality articulation and expansive range opposition (Cao *et al* 1994) [7]. The greater part of these PR proteins have antifungal action, and contribute compelling and expansive range of sickness obstruction in BjNPR1 transgenic lines. The statement of BjPR qualities in BjNPR1 transgenics further uncovers that NPR1 enacts SAR in B. juncea, a resistant reaction compelling against numerous microbes. Strikingly, microarray investigation in Arabidopsis uncovered that among SA-actuated guard qualities, over 90% were NPR1-subordinate qualities (Blanco *et al* 2009) [6]. Then again, Arabidopsis npr1 freaks are definitely not receptive to SA, are undermined in their capacity to communicate PR qualities like PR1, PR2, and PR5 (Liu *et al* 2005) [32]. Numerous reports have uncovered that NPR1 presents protection from both necrotrophs and biotrophs (Malnoy *et al* 2007) [35] which prompted the proposition of present and overexpression of BjNPR1 as a promising up-and-comer quality for designing broad spectrum sickness obstruction in B. juncea. To assess the job of BjNPR1 in sickness obstruction, two lines (L2 and L5) were picked for sickness screening against Alternaria and fine buildup contamination. As of late, overexpression of NPR1 in nut was accounted for to lead upgraded sickness obstruction against contagious microorganisms (Sunderasha *et al* 2016). Steady with these reports, the consequences of our examination uncovered that overexpression of BjNPR1 in B. juncea drives fractional illness opposition against both necrotrophic (A. brassicae) and biotrophic (E. cruciferarum) parasitic microorganism, as transgenic plants indicated deferred manifestations, decreased mean injury breadth, number of provinces and infection spreading to distal/non-tainted pieces of the plant (Figures 7, 8). These outcomes give the proof that constitutive articulation of NPR1 in B. juncea demonstrated high sharpness in distal leaves (in the type of SAR) for resulting contaminations in any event in the early phases of disease. Our outcomes likewise uncovered that overexpression of BjNPR1 postponed the beginning of Alternaria and fine mold infection accordingly shows incomplete not complete obstruction in B. juncea. Reliable with our reports, overexpression of AtNPR1 in carrot plants drove upgraded infection protection from biotrophic what's more, necrotrophic parasitic microbes (Wally *et al* 2009) [50]. Numerous investigations have indicated that NPR1 or NPR1-like proteins give opposition against parasitic and bacterial microorganisms, also, this opposition is identified with PR quality articulation in transgenic plants. In present investigation, overexpression of BjNPR1 altogether expands the record levels of SA subordinate PR qualities like BjPR1, BjPR2, and BjPR5 which are all around known to forces likely antifungal movement. Past investigations have likewise appeared that constitutive elevated level articulation of PR1, PR2, and PR5 in transgenic plants presented resilience to contamination (Gupta *et al* 2013) [25]. In synopsis, overexpression of BjNPR1 into B. juncea grants illness protection from two financially significant contagious microorganisms, accordingly supporting outcomes from past concentrates on NPR1 transgenic carrot; cotton, and nut (Parkhi *et al* 2010) [40]. Contrasts in the degree and improvement of sickness manifestations brought about by A. brassicae and E. cruciferarum between BjNPR1 what's more, untransformed plants were unmistakably watched. The illness obstruction of BjNPR1 transgenic B. juncea presented to contagious microbes might be because of the assurance presented by the collection of PR qualities and SA interceded enactment of SAR. Be that as it may, in Arabidopsis, AtNPR1 likewise is related with the actuation of fundamental guards that are autonomous of SA (Pieterse *et al* 1998) [42]. Future examinations will be completed to guarantee the general proficiency of infection opposition of overexpression of BjNPR1 transgenic plants under field conditions. Our future study would likewise zero in on investigating the part of BjNPR1 against joined biotic and abiotic stresses in B. juncea which is the topic of future examination. The outcomes portrayed in this examination highlight the need to additionally dismember the flagging pathways or knockout freak studies will give new experiences into the exact elements of the BjNPR1 quality in directing reactions to biotic just as in abiotic stress in B. juncea. For sure, constitutive articulation of AtNPR1 in numerous yields was related with predominating and the unconstrained advancement of sores which was not seen in BjNPR1 transgenic plants which could have limit their business characteristics. Notwithstanding, considering the negative aggregates related with AtNPR1 articulation in rice, the abuse of NPR1 for improving sickness obstruction in monetary significant harvests should contemplate the physiology of the transgenic plants. Taking everything into account, BjNPR1 may fill in as a potential applicant quality for creating sickness safe transgenic crops by hereditary designing. (Aravind *et al*1999) [2]

Reference

1. Ali S, Chandrashekar N, Rawat S, Nayanakantha NMC, Mir ZA, Manoharan A, *et al*. Isolation and molecular characterization of pathogenesis related PR2 gene and its promoter from Brassica juncea. Biol. Plant. 2017; 61:763-773. doi: 10.1007/s10535-017-0726-7
2. Aravind L, Koonin EV. Fold prediction and evolutionary analysis of the POZ domain: structural and evolutionary relationship with the potassium channel tetramerization domain. J. Mol. Biol. 1999; 285:1353-

1361. doi: 10.1006/ jmbi.1998.2394
3. Backer R, Mahomed W, Reeksting BJ, Engelbrecht J, Ibarra–Laclette E, van den Berg N, *et al*. Phylogenetic and expression analysis of theNPR1-like gene family from Persea americana (Mill). Front. Plant Sci. 6:300.doi: 10.3389/fpls.2015.00300, 2015.
4. Bal RS, Kumar A. Studies on the epidemiology of white rust and Alternaria leaf blight and their effect on the yield of Indian mustard. Afr. J. Agric. Res. 2014; 9:302-306. doi: 10.5897/AJAR2013.7352
5. Bari R, Jones JD. Role of plant hormones in plant defence responses. Plant Mol. Biol. 2009; 69:473-488. doi: 10.1007/s11103-008-9435-0
6. Blanco F, Salinas P, Cecchini NM, Jordana X, Van Hummelen P, Alvarez ME, *et al*. Early genomic responses to salicylic acid in Arabidopsis. Plant Mol. Biol. 2009; 70:79-102. doi: 10.1007/s11103-009-9458-1
7. Cao H, Bowling SA, Gordon AS, Dong X. Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance. Plant Cell. 1994; 6:1583-1592. doi: 10.1105/ tpc.6.11.1583
8. Cao H, Glaze brook J, Clarke JD, Volko S, Dong X. The Arabidopsis NPR1 gene that controls systemic acquired resistance encodes anovel protein containing ankyrin repeats. Cell. 1997; 88:57-63. doi:10.1016/ S0092-8674(00)81858
9. Cao H, Li X, Dong XN. Generation of broad–spectrum disease resistance by overexpression of an essential regulatory gene in systemic acquired resistance. Proc. Natl. Acad. Sci. U.S.A. 1998; 95:6531-6536. doi: 10.1073/pnas.95.11.6531,
10. Chandrashekar N, Ali S, Rawat S, Grover A. Gene expression profiling of Arabidopsis thaliana chitinase genes in response to Alternaria brassicae challenge. Indian Phytopathol. 2015; 68:106-111.
11. Chern M, Fitzgerald HA, Canlas PE, Navarre DA, Ronald PC. Overexpression of a rice NPR1 homolog leads to constitutive activation of defense response and hypersensitivity to light. Mol. Plant Microbe Interact. 2005; 18:511-520. doi: 10.1094/MPMI-18-0511
12. Dai L, Wang D, Xie X, Zhang C, Wang X, Xu Y, *et al*. The novelgene VpPR4–1 from Vitis pseudoreticulata increases powdery mildew resistancein transgenic Vitis vinifera L. Front. Plant Sci. 2016; 7:695. doi: 10.3389/fpls.2016.00695
13. De Torres–Zabala M, Truman W, Bennett MH, Lafforgue G, Mansfield JW, Rodriguez Egea P, *et al*. Pseudomonas syringae pv. tomato hijacks the Arabidopsis abscisic acid signalling pathway to cause disease. EMBO J. 2007; 26:1434-1443. doi:10.1038/ sj.emboj.7601575
14. Després C, Chubak C, Rochon A, Clark R, Bethune T, Desveaux D, *et al*. The Arabidopsis NPR1 disease resistance protein is a novel cofactor thatconfers redox regulation of DNA binding activity to the basic domain/leucinezipper transcription factor TGA1. Plant Cell. 2003; 15:2181-2191. doi: 10.1105/tpc.012849
15. De-Vos M, Van-Oosten VR, van-Poecke RMP, Van-Pelt JA, Pozo MJ, Mueller MJ, *et al*. Signal signature and transcriptome changes of Arabidopsis during pathogen and insect attack. Mol. Plant Microbe Interact. 2005; 18:923-937. doi: 10.1094/MPMI-18-0923
16. Dong X. NPR1, all things considered. Curr. Opin. Plant Biol. 2004; 7: 547-552. doi: 10.1016/j.pbi.2004.07.005
17. Durrant WE, Dong X. Systemic acquired resistance. Annu. Rev. Phytopathol. 2004; 42:185-209. doi: 10.1146/annurev.phyto.42.040803.140421
18. Dutt M, Barthe G, Irey M, Grosser J. Transgenic citrus expressingan Arabidopsis NPR1 gene exhibit enhanced resistance against huang longbing (HLB; citrus greening). PLOS ONE. 2015; 10:e0137134. doi: 10.1371/journal.pone.0147657
19. Endah R, Beyene G, Kiggundu A, van–den Berg N, Schluter U, Kunert K, *et al*. Elicitor and Fusarium–induced expression of NPR1–like genesin banana. Plant Physiol. Biochem. 2008; 46:1007-1014. doi: 10.1016/j.plaphy.2008.06.007
20. Fu ZQ, Dong X. Systemic acquired resistance: turning local infectioninto Global defense. Annu. Rev. Plant Biol. 2013; 64:839-863. doi: 10.1146/annurevarplant-042811-105606
21. Fu ZQ, Yan S, Saleh A, Wang W, Ruble J, Oka N, *et al*. NPR3 andNPR4 are receptors for the immune signal salicylic acid in plants. Nature. 2012; 486:228-232. doi: 10.1038/nature11162
22. Giri P, Taj G, Kumar A. Comparison of artificial inoculation methods for studying pathogenesis of Alternaria brassicae (Berk.) Sacc on Brassica juncea (L.) Czern. (Indian mustard). Afr. J. Biotechnol. 2013; 12:2422-2426.
23. Glazebrook J. Contrasting mechanisms of defense against biotrophic andnecrotrophic pathogens. Annu. Rev. Phytopathol. 2005; 43:205-227. doi:10.1146/ annurev.phyto.43.040204.135923
24. Glazebrook J, Rogers EE, Ausubel FM. Isolation of Arabidopsismutants with enhanced disease susceptibility by direct screening. Genetics. 1996; 143:973-982.
25. Gupta P, Ravi I, Sharma V. Induction of b-1, 3-glucanase andchitinase activity in the defense response of Eruca sativa plants againstthe fungal pathogen Alternaria brassicicola. J. Plant Interact. 2013; 8:155-161.doi: 10.1080/17429145.2012.679705
26. Holsters M, Waele D, Depicker A, Messens E, van Montagu M, Schell J, *et al*. Transfection and transformation of Agrobacterium tumefaciens. Mol. Gen. Genet. 1978; 163, 181-187. doi: 10.1007/BF00267408
27. Kinkema M, Fan W, Dong X. Nuclear localization of NPR1 isrequired for activation of PR gene expression. Plant Cell. 2000; 12:2339-2350. doi: 10.1105/tpc. 12.12.2339
28. Koornneef A, Pieterse CM. Cross talk in defense signaling. Plant Physiol. 2008; 146:839-844. doi: 10.1104/pp.107.112029
29. Kunkel BN, Brooks DM. Cross talk between signaling path waysin pathogen defense. Curr. Opin. Plant Biol. 2002; 5:325-331. doi: 10.1016/S1369-5266(02)00275-3
30. Le Henanff G, Farine S, Kieffer-Mazet F, Miclot AS, Heitz T, Mestre P, *et al*. Vitis vinifera VvNPR1.1 is the functional ortholog of AtNPR1 andits overexpression in grapevine triggers constitutive activation of PR genes andenhanced resistance to powdery mildew. Planta. 2011; 234:405-417. doi: 10.1007/s00425-011-1412-1
31. Li J, Brader G, Palva ET. The WRKY70 transcription factor: a nodeof convergence for jasmonate-mediated

and salicylate-mediated signals in plant defense. Plant Cell. 2004; 16:319-331. doi: 10.1105/tpc.016980
32. Liu G, Holub EB, Alonso JM, Ecker JR, Fobert PR. An Arabidopsis NPR1-like gene, NPR4, is required for disease resistance. Plant J. 2005; 41:304-318. doi: 10.1111/j.1365-313X.2004.02296x
33. Livak KJ, Schmittgen TD. Analysis of relative gene expressiondata using real–time quantitative PCR and the 2??11CT method. Methods. 2001; 25:4402-4408. doi: 10.1006/meth.2001.1262
34. Makandar R, Essig JS, Schapaugh MA, Trick HN, Shah J. Genetically engineered resistance to Fusarium head blight in wheatby expression of Arabidopsis NPR1. Mol. Plant Microbe Interact. 2006; 19:123-129.doi: 10.1094/MPMI-19-0123
35. Malnoy M, Jin Q, Borejsza-Wysocka EE, He SY, Aldwinckle HS. Overexpression of the apple MpNPR1 gene confers increased disease resistance in Malus x domestica. Mol. Plant Microbe Interact. 2007; 20:1568-1580.doi: 10.1094/MPMI-20-12-1568
36. Mazumder M, Das S, Saha U, Chatterjee M, Bannerjee K, Basu D, *et al*. Salicylic acid mediated establishment of the compatibility between Alternaria brassicicola and Brassica juncea is mitigated by abscisic acid in Sinapis alba. Plant Physiol. Biochem. 2013; 70:43-51. doi: 10.1016/j.plaphy.2013.04.025
37. Mou Z, Fan W, Dong X. Inducers of plant systemic acquiredresistance regulate NPR function through redox changes. Cell. 2003; 113:935-944. doi:10. 1016/S0092-8674(03)00429-X
38. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant. 1962; 15:473-497. doi:10.1111/j.1399-3054. 1962.tb08052.x
39. Oliver RP, Ipcho SVS. Arabidopsis pathology breathes new life intothe necrotrophs-vs.-biotrophs classification of fungal pathogens. Mol. Plant Pathol. 2004; 5:347-352. doi: 10.1111/j.1364-3703.2004.00228.x
40. Parkhi V, Kumar V, Campbell LM, Bell AA, Shah J, Rathore KS, *et al*. Resistance against various fungal pathogens and reniform nematodein transgenic cotton plants expressing Arabidopsis NPR1. Transgenic Res. 2010; 19:959-975. doi: 10.1007/s11248-010-9374-9
41. Pieterse CM, Van Loon LC. NPR1: the spider in the web ofinduced resistance signaling pathways. Curr. Opin. Plant Biol. 2004; 7:456-464. doi:10.1016/j.pbi. 2004.05.006
42. Pieterse CM, van Wees SC, van Pelt JA, Knoester M, Laan R, Gerrits H, *et al*. A novel signaling pathway controlling induced systemic resistancein Arabidopsis. Plant Cell. 1998; 10:1571-1580. doi:10.1105/tpc. 10.9.1571
43. Rochon A, Boyle P, Wignes T, Fobert PR, Després C. Theco activator function of Arabidopsis NPR1 requires the core of its BTB/POZdomain and the oxidation of C–terminal cysteines. Plant Cell. 2006; 18:3670-3685.doi: 10.1105/tpc.106.046953
44. Roux F, Voisin D, Badet T, Balagué C, Barlet X, Huard-Chauveau C, *et al*. Resistance to phyto pathogens e tutti quanti: placing plant quantitative disease resistance on the map. Mol. Plant. Pathol. 2014; 15:427-432. doi: 10.1111/mpp.12138
45. Sharma MK, Solanke AU, Jani D, Singh Y, Sharma AK. A simple and efficient Agrobacterium–mediated procedure for transformation of tomato. J. Biosci. 2009; 34:423-433. doi: 10.1007/s12038-009-0049-8
46. Spoel SH, Koornneef A, Claessens SM, Korzelius JP, Van-Pelt JA, Mueller MJ, *et al*. NPR1 modulates cross–talk between salicylate–and jasmonate–dependent defense pathways through a novel function in thecytosol. Plant Cell. 2003; 15:760770.doi:10.1105/tpc.009159
47. Sundaresha S, Rohini S, Appanna VK, Arthikala MK, Shanmugam NB, Shashibhushan NB, *et al*. Co-overexpression of Brassica juncea NPR1 (BjNPR1) and Trigonella foenum-graecum defensin (Tfgd) in transgenic peanut provides comprehensive but varied protection against Aspergillus flavus and Cercospora arachidicola. Plant Cell Rep. 2016; 35:1189-1203. doi: 10.1007/s00299-016-1945-7
48. Tada Y, Spoel SH, Pajerowska-Mukhtar K, Mou Z, Song J, Wang C, *et al*. Plant immunity requires conformational charges of NPR1 viaS-nitrosylation and thioredoxins. Science. 2008; 321:952-956. doi: 10.1126/science.1156970
49. Thakur R, Kolte SJ. Radish root extract agar, a suitable medium forthe growth and sporulation of Alternaria brassicae. News Lett. 1985; 10:117-118.
50. Wally O, Jayaraj J, Punja ZK. Comparative resistance to foliar fungal pathogens in transgenic carrot plants expressing genes encoding forchitinase, beta–1, 3–glucanase and peroxidase. Eur. J. Plant Pathol. 2009. 2009; 123:331-342. doi: 10.1007/s10658-008-9370-6
51. Yuan Y, Zhong S, Li Q, Zhu Z, Lou Y, Wang L, *et al*. Function alanalysis of rice NPR1-like genes reveals that OsNPR1/NH1 is the rice ortho logue conferring disease resistance with enhanced herbivore susceptibility. Plant Bio technol. J. 2007; 5:313-324. doi: 10.1111/j.1467-7652.2007.00243.x
52. Zhang Z, Wang M, Li Z, Li Q, He Z. Arabidopsis GH3.5 regulatessalicylic acid–dependent and both NPR1– dependent and independent defense responses. Plant Signal. Behav. 2008; 3:537-542. doi:10.4161/ psb.3.8.5748