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Towards development of a universal dengue vaccine – How close are we?

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ABSTRACT

Dengue has been ranked as one of the top emerging diseases in Asia and Latin America. Current epidemiological data may not even reflect the true burden of disease due to under-reported figures. Vector control programmes have failed to contain the disease and worst of all, no specific treatment is available at the moment. Thereby, this pushes the demand for a dengue vaccine as a long-term protective approach. Despite there are numerous vaccine candidates ahead, they could be held back by different aspects in promoting vaccine implementation. Particularly for developing nations, logistics and cost are the major hurdles that need to be addressed in order to provide a quick yet affordable medical relief. As an alternative, plant-based vaccine production system is able to offer an attractive prospect given to its advantages of biocontainment warranty, low operation cost, rapid scalability and logistics flexibility. Researches that have embarked on this scope are laid out and reviewed in terms of the feasibility of plant system to serve as a biofactory for dengue vaccine.

1. Introduction

Dengue viral infection is currently labelled as a fast-emerging tropical disease that has drawn increasing public concern in recent years. Up to date, transmission of dengue virus (DENV) has been reported in more than 128 countries, primarily affecting tropics and subtropics of the Asia and Latin America regions [1]. The disease severity can range from undifferentiated acute febrile, classical dengue fever (DF) to life-threatening manifestations such as dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) [2]. Individual encounters the disease for the first time usually will have asymptomatic or non-severe dengue. This primary infection results in a life-long immunity against that particular serotype, and a transient cross-protection that lasts for 3–4 months [3]. Beyond the cross-immunity period, a secondary infection with another subtype is known to be exposed to higher risk of severe dengue [4]. Such aggravation is believed to be associated with the complexity of human immune system, known as antibody dependent enhancement (ADE) [5]. Annual incidence rate has grown dramatically high in recent decades; as compared to previous figures of 50–100 million reported DF cases and 250 000–500 000 patients hospitalised with DHF and DSS [6]. In fact, recent data gave a startling estimate of dengue burden that tripled past predictions, mapping 390 million infections per annum instead [7]. Furthermore, it was projected that over 5–6 billion of world populace may be exposed to dengue transmission by 2080s, attributed by climate change and population growth [8].

Being classified as one of the members of *Flaviviridae* family, DENV is a 500 Å plus-sense RNA virus with the electron-dense core surrounded by a relatively smooth surface of lipid bilayer [9]. The non-segmented genome is 10.862 kilo-base pairs (kbp) long, encodes for a single polypeptide that comprised of the structural proteins [capsid (C); envelope glycoprotein (E); precursor membrane (prM)] and non-structural biomolecules (NS1, 2A, 2B, 3, 4A, 4B and 5) [10]. In fact, the three structural blocks exist in stoichiometric quantities within the 50 nm particle (C, 100 amino acids; E 495 amino acids; prM, 75 amino acids) and give rise to the icosahedral symmetry [9]. Often regarded as the major antigenic determinant, DENV E protein is divided into three functional domains (DI-III) which

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plays a central role to facilitate viral attachment and entry into host cells [11].

Dengue mainly prevails as four different serotypes (DENV1-4), where geographical expansion of different subtypes had been remarkably apparent since 1980s [12]. First isolation of the virus was documented in 1943 after an epidemic outbreak occurred in Japan [13]. Thereafter, disease transmission has followed the spread of the vector mosquitoes *Aedes aegypti* (*Ae. aegypti*), and to a lesser extent, *Aedes albopictus*. It was pointed out that DENV could be maintained between epidemics by overwintering in vectors, vertically passed in mosquitoes and persisted via silent transmission given the high number of asymptomatic cases [14]. The incidence of reported DHF/DSS has now expanded by more than 500 folds since World War II [15], spreading from Southeast Asia into the Americas and Western Pacific regions. Nonetheless, actual scenario could be worse than reported figures which presumed an under-representation. Murray *et al* had reviewed multi-factors that could drive the dengue activity; including evolution of highly-virulent virus, proliferation of climate-dependent *Aedes* vectors, increased passenger travel and cargo trading, unplanned urbanisation as well as unprecedented socioeconomic development [16]. To address the continued expansion, there is a need to devise an active epidemiological surveillance and invest efforts on more effective vector control.

2. Current prevention and control strategies

Mosquito control remains as one of the most adopted approaches to curb dengue incidence due to the lack of effective treatment or vaccine. This mainly relies on source reduction, where interventions are aimed at minimising the oviposition sites. Law enforcement and public education are also important drivers to help in reducing vector population [17]. Prevention has become increasingly challenging as *Aedes* mosquitoes are circulating in close contact with humans and most major cities are populated with 15–20 million people [18]. Global urban population has grown so rapidly since 1950s and it is projected that the continuous population shift will raise the percentage to 66% by 2050, making a reversal to the rural-urban distribution in mid-20th century [19]. Prevention could be an uphill task by then, if community engagement is not strongly advocated. Moreover, eradication programmes in most endemic countries have ambiguous goals with diluted commitments that are initiated only during the time of epidemics [20]. World Health Organization, WHO (2012a) also stressed that successful control must be centred between vigilant monitoring and sustainable inventions in order to achieve a significant impact [21]. An integrated vector management (IVM) approach had been introduced by WHO (2012b) to implement a cost-effective, sustainable and ecological sound vector control via the optimal use of local resources and existing systems [22]. To date, dengue containment attempts are ranged from the conventional chemical application to innovative biological control.

For decades, insecticidal deployment has been difficult to sustain due to the high cost and limited effectiveness. In the 1940s, *Ae. aegypti* vector control was initiated by Pan American Health Organization (PAHO) as an effort to avert urban epidemics of yellow fever [23]. It was a great success as the vector was largely eliminated from 73% of initially infested areas through the use of dichloro-diphenyl-trichloroethane (DDT) [24]. That being so, the programme was terminated since there was no perceived need to carry on such expensive system. Situation was exacerbated by deterioration of surveillance and slow response to re-infestations [25]. By 1970s, most of these countries suffer from vector re-invasion and DENV continues to circulate up till alarming pandemics at this stake. Ironically, the disease went from virtually non-existence into one of the region's major public health problems just within a flash of 20 years [26]. At present, different insecticide classes (i.e. organophosphates and pyrethroids) are applied in rotation to preclude an emergence of resistance in *Ae. aegypti* mosquitoes. Unregulated treatment not only induces a spread of resistance that arises from structural alteration of insecticide target sites or elevated detoxifying enzymes activities, but the trace of chemical residues may also affect human and environmental health [27].

In terms of biological intervention, the use of *Wolbachia* transinfection in *Ae. aegypti* has also gained increasing attention nowadays. These cytoplasmically inherited bacteria are able to infect the ovaries and testes of many arthropod species and alter the host reproduction through cytoplasmic incompatibility, feminisation and parthenogenesis [28]. DENV transmission can be suppressed by sabotaging the vector breeding cycle and *Wolbachia* also blocks viral replication in salivary glands of mosquitoes [29]. On the other hand, a sterile male *Ae. aegypti* (OX513A) was genetically modified (GM) to harbour a dominant, repressible and late-acting lethal transgene insertion, which can be differentiated through the expression of red fluorescence [30]. The study reported that, in the absence of tetracycline, high expression of the lethal factor could limit survival of transgenics by 95–97% at late-larval or early-pupal stage. Nonetheless, the abovementioned biological approaches are still being assessed via field trials in order to warrant a long-term effectiveness. Implementation of any new programme should prudently integrate public engagement beforehand based on the backlash experienced for GM mosquitoes release in Malaysia [31].

3. Longing for an effective dengue vaccine

The call for dengue vaccine development is an endless priority; this is not only driven by the lack of dengue-specific drugs and treatments, but also the inconsistent efficacy of current vector control regimes. Over 70 years have passed since the isolation of dengue virus and no vaccine is yet available. Progress is delayed by hurdles which are unique to the disease, such as (1) co-circulation of multiple serotypes with unpredictable predominance at different time points, (2) gap in understanding the viral pathogenesis, (3) lack of a reliable animal model; and (4) complexity of host immunological mechanisms [32]. As secondary infection is often associated with higher risk of severe dengue, a vaccine must provide a long-term protection against all dengue virus serotypes simultaneously. These challenges, along with the absence (almost) of dengue in developed countries have rendered vaccine development less appealing to the industry throughout most of the 20th century. The longing hope for a dengue vaccine has only been re-instilled through a big leap of achievements reported in recent years. Vaccine candidates that either have completed or been undergoing clinical trial stages are laid out in Table 1, with the earliest licensure time as predicted by Mahoney [33]. In fact, the Pediatric Dengue Vaccine Initiative founded in 2001 aims to accelerate the

Table 1
Current dengue vaccine candidates that are being examined in various stages of clinical trials.

Vaccine	Type	Stage	Earliest Licensure[a]
CYD-TDV Sanofi Pasteur	Live attenuated chimeric	Completed	2015
TV003/TV005 (NIH/NIAID/Butantan Institute)	Live attenuated chimeric	Phase III	2018–2019
TDV (Inviragen/Takeda)	Live attenuated chimeric	Phase III	2017–2018
TDEN (GSK/WRAIR)	Purified inactivated whole virus	Phase II	2018
V180 (Merck/NIAID)	Subunit	Phase I	–
D1ME100 (U.S. Naval Medical Institute)	DNA	Phase I	–

[a] Source: Mahoney (2014) [33].

research pace and brings these promising candidates into realisation [34].

3.1. Live attenuated tetravalent vaccine

After spanning decades of efforts, the world first dengue vaccine had been launched by Sanofi Pasteur in November 2014, branded as Dengvaxia®. The ChimeriVax Technology was first developed by St. Louis University to generate a molecular clone of Yellow Fever Virus (YFV) 17D strain with Japanese Encephalitis Virus (JEV) structural proteins [35]. The C protein however was excluded as the chimera failed to recover, attributed to incompatibility of *cis*-acting RNA elements, inefficient protease processing on JEV C/prM junction or defectiveness in viral replication and packaging. This chimeric virus was then tested in mice and demonstrated an effective protection profile [36]. With the successful prototype, Guirakhoo *et al* went on engineering the first YFV 17D/DENV2 chimera that harboured heterologous prM and E proteins [37]. Subsequent efforts focused on the construction of tetravalent YF/DENV1-4 using wild-type genes from low-passage human isolates of DENV, and the chimeras generated from RNA-transfected Vero cells were tested in non-human primates as monovalent and tetravalent formulations [38]. Despite tetravalency protection was displayed, the higher activity of YF/DENV2 demanded for further refinement where reconstruction of mutated viruses, plaque purification and dose adjustment were done [39,40]. With that, the finalised tetravalent formulation [i.e. equivalent to 5 log plaque forming units (PFU) of each serotype] was tested in a Phase 1 trial. Referred as chimeric-yellow fever-dengue (CYD), the vaccine was shown to be well-tolerated with full seroconversion among flavivirus-naïve adults; but it was found that the first dose mainly induced responses against DENV2 and DENV4, hence a prolonged immunisation schedule was proposed in order to limit a short-term viral interference [41].

Other candidate in the pipeline includes the vaccine co-developed by the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) and Butantan Institute. Preliminary work was started with the virus attenuation via 3′ UTR deletion mutations or chimerisation with genome of other serotype, and subsequent assessment resulted in the selection of most favourable monovalent vaccines [42]. Different admixtures of the pre-selected monovalent components were then tested in the Phase 1 trial, of which TV003 had been identified as the best tetravalent candidate giving 90% of seropositivity after a single dose [43]. However, the vaccine also obtained a relatively weaker seroconversion against DENV2 like CYD. Further optimisation was done by Kirkpatrick *et al* [44]. It was shown that TV005 (with increased DENV2 dose) afforded a higher immunogenicity when compared to TV003, but a single dose of either one was sufficient to impart a sterilising immunity. In addition, TV003 protective efficacy was re-assessed in a DENV2-challenge model among the healthy volunteers, and a complete protection was observed without signs of rash, neutropenia and thrombocytopaenia [45]. Another challenge studies for TV005 is ongoing with a Phase 3 trial scheduled to embark in early 2016 to assess for the long-term efficacy of TV003 [46].

Takeda to which had acquired Inviragen, is also evaluating its tetravalent vaccine candidate with the registration name as TDV (formerly known as DENVax). The vaccine virus was developed based on the live-attenuated DENV2 strain designated as PDK-53, which was then used as the genetic background for the other three chimeric viruses by replacing the prM and E proteins with wild-type DENV1, DENV3 and DENV4 correspondingly [47]. Albeit Phase 1 clinical trials showed that TDV did induce neutralising antibody response against all serotypes, but the percentage of flavivirus-naïve adults who acquired tetravalent protection varied between 46% and 80% [48]. It was most likely due to a lower seroconversion against DENV-4, and therefore concentration of TDV-4 in the 'high-dose' TDV formulation was increased in order to boost up an overall protection [49]. Meanwhile, results from the Phase 2 clinical trial indicated that TDV induced a neutralising antibody against all serotypes and the vaccine was well-tolerated in all age groups irrespective of pre-vaccination dengue serostatus [50]. One more Phase 2 study that assesses immunogenicity of TDV among paediatric volunteers living in dengue endemic countries is still active [51]. Just recently, Takeda has announced the embarkation of Phase 3 clinical trial that will enrol 20 000 healthy children to study for the efficacy of TDV in preventing symptomatic DF of any severity [52].

3.2. Inactivated whole virus vaccine

One of the earlier events of dengue vaccine development came from the attenuation of DENV by intracerebral inoculation in mice [53]. From the initial research on mouse brain vaccine production, the United States Army had then replaced it with safer propagation in cell cultures [54]. This project led by Walter Reed Army Institute of Research (WRAIR), did not yield a success due to varying levels of attenuation and immunogenicity exhibited by each serotype. Serial passages in primary dog kidney cells then offered a better alternative whereby candidates for Phase 1 human trials were selected after evaluating the infectivity in rhesus monkeys [55]. These monovalent candidates were assessed in a series of trial runs to develop a potential tetravalent formulation [56,57]. In fact, a total of 16 tetravalent preparations were assessed by Edelman *et al* (2003) by mixing the lyophilised monovalent components in different dose combinations [58]. All of the studies mentioned thus far were completed in 2000 and since then, further clinical assessment was conducted in

collaboration with GlaxoSmithKline (GSK) [54]. Phase 2 trials expanded the evaluation of selected formulations and the best candidate with acceptable balance of reactogenicity and immunogenicity was identified, conferring 63% of tetravalent neutralisation after administration of two doses at 6 months apart [59]. Subsequent paediatric study with flavivirus-naïve children indicated that the vaccine was well-tolerated with 100% of tetravalent protection acquired one month after the second dose [60]. Similar testing on seronegative Thai infants demonstrated the absence of serious adverse events, but with lower tetravalent seroconversion of 53.5% [61]. Thomas *et al* then re-derived a new vaccine candidate (referred as TDEN) by subjecting a precursor strain to additional passages in fetal rhesus lung cells, formulating the monovalent components with carbohydrate stabiliser and lyophilising the final product in a tetravalent form [62]. Clinical profile of the new candidate was shown to be safe for administration in healthy adults, despite not much differences were observed when compared to the precursor vaccine alongside.

3.3. Subunit protein vaccine

Initial efforts were made by Hawaii Biotech to express dengue recombinant E protein in yeast and mammalian cells, however, all these works did not yield a significant success until the *Drosophila* Schneider-2 (S2) cell expression system was adopted [63]. More specifically, the truncated subunit protein (DEN-80E) was secreted by transformed S2 cells to express full length prM sequence and 80% of amino-terminal of E molecule [64]. Purified 80E subunits of each serotype were then combined and adjuvanted with ISCOMATRIX® for immunogenicity testing in animal models. Neutralisation against all serotypes had been shown, but seroconversion was not equivalent as seen from the lower anti-DENV4 response. Further attempts were explored by Govindarajan *et al* (2015) to improve the immunogenicity of the tetravalent vaccine, particularly on DEN4-80E through dosage adjustment, vaccination schedule and choice of adjuvant [65]. The overall findings attested superiority of the subunit proteins vaccine since administration of low antigen dose (ISCOMATRIX®-adjuvanted) was able to confer a balanced protection in rhesus monkeys. Through collaborative efforts of Merck and NIAID, the vaccine candidate (V180) has recently completed the Phase 1 clinical trials for evaluation of its safety and immunogenicity profiles in healthy adults [66,67].

3.4. DNA vaccine

On the other hand, U.S. Naval Medical Research Institute has ventured far into the less-adopted DNA vaccine technology. It first started with the construction of a few recombinant plasmids expressing different structural segments of DENV1 and each construct was then injected into mice to test for an optimal neutralising response [68]. The ME100 construct that harboured prM gene along with the full length E sequence was chosen for further testing based on the best neutralisation activity. Partial to complete protection was observed when immunised rhesus macaques were subjected to a viral challenge [69]. Several strategies had also been tested in order to boost up the vaccine efficacy. One of them was based on the gene localisation approach, where the mouse lysosome-associated membrane protein (LAMP) targeting signal was used to replace the transmembrane and carboxy-terminal of dengue E gene [70]. As a result, LAMP directed the trafficking of DENV2 chimera antigen to major histocompatibility complex (MHC) II pathway and induced a higher neutralisation titre in mice as compared to the original construct. In addition, co-immunisation of the DNA vaccine with human immunostimulatory sequences and *Aotus* cytokine gene using needle-free Biojector® had generated a superior immune response in primate studies [71]. A prototype testing of DENV1 vaccine candidate (D1ME100) was then conducted among flavivirus-naïve adults in the Phase 1 clinical trial [72]. Unexpectedly, only those who received high dosage developed anti-dengue response but it was merely less than 50% of the subjects. A breakthrough had been achieved when the tetravalent dengue DNA vaccine (containing equal mixture of serotype-specific plasmid DNAs) was formulated with Vaxfectin®, as evidenced by a greater anti-dengue neutralising response in primates [73]. The adjuvanted vaccine was also tested in New Zealand white rabbits and data indicated that it was well-tolerated with 100% of neutralisation against all serotypes [74]. Though DNA vaccine is comparably stable, easy to prepare, modify and/or scale-up, which these also do not involve high purification cost, but the ability to stimulate robust and durable protection still remains to be determined.

4. Current issues in adoption of Dengvaxia® vaccine

A number of reviews have argued that serotype interferences represent a critical issue that need to be addressed by Sanofi Pasteur. Imbalance viral replication of the four monovalent serotypes along with epitopes-linked immunodominance had been observed when the vaccine was administrated as tetravalent formulation [75]. Furthermore, the Phase 2b clinical study also highlighted that CYD could not afford any protection against DENV2 [76]. Based on the Phase 3 results, the vaccine had only conferred a modest protection to dengue-naïve individuals in Asia (56.5%) and Latin America (60.8%), with the efficacy against DENV2 still being the lowest (35% and 42.3%, respectively) [77,78]. Apparently, the efficacy is far less than the minimum acceptable level, i.e. 80% against four serotypes, as demanded by policy-makers and influential professionals in Southeast Asian countries [79]. In the long run, more conclusive data on its tetravalency protection need to be assessed meticulously to guarantee safe and durable use. This is also considering that DENV2 is often associated with an outbreak of severe dengue. The dose administrations which are set apart at 0, 6 and 12 months, call for a more extended schedule than most licensed vaccines and this might impose problem in terms of compliancy [33]. Moreover, the reduced efficacy for dengue-naïve subjects at first vaccination presents one of the major hiccups for CYD implementation [46]. A long-term post-hoc analysis also indicated that the risk of hospitalisation was higher among CYD paediatric recipients in their third year post-vaccination as compared to placebo control [80]. Thus, Sanofi vaccine can only be administered to individuals aged between 9 and 45 years in endemic areas [81]; and this actually signifies that a universal vaccine is still not available yet. Up to date, only four countries have granted market access to Sanofi's Dengvaxia® including Brazil, Mexico, Philippines and Singapore. On the whole, the partial protection

offered by CYD has most likely limited its global adoption due to these uncertainties.

5. Molecular pharming–an alternative to derive the next generation vaccine

For centuries, plants have been extensively sought by mankind for food, medicine, fuel and shelter. The traditional way of extracting useful proteins or metabolites from the natural biodiversity are delimited by various factors, until the advent of modern biotechnology. This is achieved through using tools of recombinant DNA to facilitate an exchange of genetic material. The pressure from growing clinical demand and limitations of established systems has spurred an interest in utilising plant as the 'green factory' for bio-production. Molecular techniques can now be applied to synthesise commercially valuable products using plant system, either by manipulation of biosynthetic pathways or alteration to desired-features [82]. This has then sparked the idea of 'Molecular Pharming' to harness the power of agriculture to produce useful pharmaceutics and industrial proteins (i.e. antibodies, vaccines and therapeutic enzymes). As stated by Whitelam *et al*, plant has emerged as a convenient, safer and economical alternative that cannot be matched by many existing production platforms [83]. Plant system generally offers the advantages of low production cost, flexible scalability, proper protein folding and glycosylation, free from endotoxins and human pathogens along with ease of storage and distribution.

In molecular biology, transformation is described as the alteration of genetic constituent resulting from uptake, integration and expression of a foreign DNA. The history of plant transformation actually began in 1983, of which a few research groups had successfully introduced bacterial genes into plant genome [84–86]. Stable nuclear transformation ultimately facilitates the establishment of plant lines with stably inheritable traits which can be passed on via Mendelian inheritance [87]. This can be achieved via vector-based (e.g. *Agrobacterium*-mediated) and/or direct gene transfer methods (e.g. biolistic). Plastid transformation was introduced in the 1980s as an alternative to nuclear transformation [88]. Commercially, stable transformants are valued for the inherent scalability and permanent establishment of superior lines that facilitate cost-effective utilisation of large acreage [89]. Transient assay, on the other hand, refers to short-term expression of transgene which is not inherited by the germ line. Initial works on transient assay was done by leaf disc transformation, where analysis of transformation efficiency can be performed two days after the co-culture [90]. Janssen and Gardner (1990) reported that gene transfer of transiently expressed β-glucuronidase (GUS) was 1 000-fold more efficient than stable integration [91]. Since then, transient assay has gained wider adoption as a platform to achieve rapid production of recombinant proteins within a matter of days. Technically, it eliminates the need for laborious handling of tissue culture, as well as the redundancy to regenerate transformants that subject to long lead time, somaclonal variation, positional effect or may even be recalcitrant to regeneration protocol [92]. Particularly for vaccine production, it enables fast screening of candidates in prompt response to an emerging outbreak. Industrial processes have been deliberately optimised for scaling-up production to commercial level that can outcompete the stable transformation [93]. Nowadays, transient expression is mostly achieved via virus vector-based or *Agrobacterium* infiltration system that combines the benefits of speed and convenience as compared to the transgenic expression [94].

6. Prospects of plant-based dengue vaccine development

Despite there are numerous vaccine candidates ahead in the pipeline, they could be held back by several challenges including vaccine efficacy (long lasting and balanced seroconversion to all four serotypes), safety profile (no vaccine-related adverse events and sensitisation for DHF), product yield (adequate supply and timely delivery to risk-prone populations), vaccine price (affordable vaccination cost particularly for developing countries), supply chain the need for less costly alternative to "cold chain", dosing schedule (shorter immunisation intervals which also fit traveller and military uses). Particularly for developing nations, cost is the major determinant that affects the vaccine uptake and utilisation. As a matter of fact, the primary occurrence of dengue fever among low- to middle-income nations does make it sensible to opt for a comparably cost-effective vaccine production platform. A survey among key decision-makers in Southeast Asia stated that governments could afford to pay a price $0.50–$1.00 per dose of dengue vaccine given to the urgent need [79]. However, this was not the case as Sanofi charged a high cost of €20 (around $20–25) per shot for the children vaccination programme in Philippines [95]. Following Dengvaxia®, TV003/TV005 will most likely be the next vaccine to advance to the finish line in near future. Economic analysis on this vaccine candidate estimated that by producing 15–60 million doses per year, the production cost would incur a price of $0.69–$1.75 per dose in single-dose vials and $0.19–0.65 in 10-dose vials [96]. But it is still uncertain whether the final delivered price would absorb additional charges that may impede the uptake by low-income nations. Moreover, a manufacturing capacity of 60 million annual doses is unlikely sufficient to meet the global demand. This is similar to the constraint as encountered by Sanofi, which can only offer 100 million doses for the first vaccine [97]. This actually signifies that current production is unlikely adequate to vaccinate 3 billion of people who are living in dengue-prone areas. As flaviviruses are notoriously unstable, formulated vaccine (e.g. yellow fever 17D) needs to be lyophilised to facilitate logistics at 2–8 °C, and the vaccine loses potency so rapidly upon reconstitution which must be discarded after an hour [98]. Such requirement for a cold chain could impose further constraints to resource-limited regions, hence, the availability of a vaccine which is stable at ambient temperature could be more ideal [32].

Cost and implementation advantages have greatly extended the utilisation of plant as a biofactory for pharmaceuticals production at agricultural scale. This would revolutionise the accessibility to many live-saving vaccines which can be tailored according to regional needs to provide rapid yet affordable medical relief. As compared to conventionally-produced vaccines, the total costing for plant production could benefit from 31% of price reduction [99]. Such cost difference might seem to be insignificant for high-income industrialised nations, but it is marginally adequate to save more lives of the impoverished one due to budgetary constraints. Albeit the concept of edible vaccine might still be far from realisation, but there is a prospect of delivering heat-stable oral vaccine such as the current polio

Table 2
List of plant-based dengue vaccine development researches.

Host Plant	Transformation approach	Expressed antigen	Tested in animal	Reference
Nicotiana benthamiana	Transient - Virus infection	DENV2-specific E Protein Domain III (EDIII)	Yes	[102]
Nicotiana benthamiana	Transient - Agroinfiltration	DENV2-specific (i) Truncated E; (ii) C/prM/E truncated; (iii) EDIII fusion to Hepatitis B core antigen	No	[103]
Nicotiana benthamiana	Transient - Agroinfiltration	(i) Consensus EDIII (cEDIII); (ii) cEDIII fusion to M-cell targeting ligand (Co1)	No	[104]
Nicotiana tabacum	Transgenic - *Agrobacterium*	DENV2-specific EDIII	No	[105]
Nicotiana tabacum	Transgenic - *Agrobacterium*	DENV2-specific EDIII	No	[106]
Nicotiana tabacum	Transplastomic - Biolistic	Tetravalent fusion of EDIII	No	[107]
Oryza sativa	Transgenic - Biolistic	cEDIII	No	[108]
Oryza sativa	Transgenic - Biolistic	cEDIII-Co1	No	[109]
Oryza sativa	Transgenic - Biolistic	cEDIII-CTB	No	[110]
Oryza sativa	Transgenic - Biolistic	DENV2-specific E	No	[111]
Zea. mays	Transgenic - *Agrobacterium*	DENV2-specific (i) EDIII; (ii) EDIII-CTB	No	[112]
Solanum tuberosum	Transgenic - *Agrobacterium*	DENV2-specific CTB-EDIII	No	[113]
Lactuca sativa	Transplastomic - Biolistic	DENV3-specific prM/E	No	[114]
Cucumis melo	Transient - Virus infection	DENV2-specific truncated E	No	[115]
Cucurbita pepo	Transient - Virus infection	DENV2-specific EDIII	No	[116]

vaccine. Oral delivery is considered as a more realistic approach to reach out for mass immunisation of deprived communities, where accessibility to infrastructure with trained personnel or sterile injection equipment may not even exist. Since the pioneer work done on plant-based vaccine [100], there is now wide adoption as proven by a number of human vaccines which are undergoing clinical trials and close to be marketed within this decade [101]. Nevertheless, research on plant-based dengue vaccine is considered relatively new in the field, with less than 20 papers reported thus far (refer to Table 2). The prime candidate antigens that have been studied are the dengue structural blocks, prM and E as well as NS1 protein. Among all, only Saejung *et al* (2007) had tested the construct in mice and reported the successful induction of anti-dengue neutralising antibody [102]. Immunogenicity for other candidates, however, is still remained to be tested. On the whole, this has definitely paved the way towards production of plant-based dengue vaccine with a global significance.

7. Conclusion

Dengue elimination represents a missionary battle of the 21st century, with approximately 40% of the world population is exposed to the risk of infection. It is a general consensus that effective vector control must be accompanied with the introduction of a safe and protective dengue vaccine. Although there are a handful of candidates in the pipeline, with a few more expected to be commercialised in near future, on-going researches are still essential to ensure a maximum outreach to the needy and poorer regions of the world. Considering the benefits of plant-based production system, attention should be drawn to its potential to displace the conventional platforms in the long run. If a plant-based dengue vaccine is proven to be successful, it will be a momentous impact in terms of revolutionising the way where vaccine can be delivered. This will be particularly beneficial in providing an affordable medical solution for diseases that engulf the developing countries.

Conflict of interest statement

We declare that we have no conflict of interest.

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