Ebola Virus: Updates on Plant Made Vaccine Development

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Abstract: **- Ebola hemorrhagic fever is a major health issue due to the lack of any approved medicine or vaccine for human use. Ebola virus disease has also spread a serious terrific fear attack in Africa since the Ebola virus disease has killed approximately 5,000 people in 2014 outbreak in West Africa. In case, if Ebola virus disease is not controlled, then it will be a serious threat to human population and also Ebola virus could be used as a bioterrorism agent which is still more dangerous and worst than the warfare. This emergency situation of Ebola virus outbreak has cautioned and also motivated for the development of a vaccine against highly contagious Ebola virus disease. However, there are many issues with the traditional methods of vaccine production. Therefore, plant made vaccine strategy coupled with the use of viral vectors (MagnICON' vectors, Icon Genetics Inc., Germany), and *Agroinfection* technique is safe, versatile, and accumulates higher yield of recombinant protein for vaccination programmes. Recent experimental model of tobacco plant derived ZMapp antibody cocktails is another major breakthrough in plant biotechnology. This new wave of ZMapp antibody cocktail experiment is another ray of hope for humanization of vaccine platform. However, there are some regulatory issues and needs to be addressed before the commercialization of vaccine.**

Key words: **Biomedicines, Green farming, infectious diseases, immunization, vaccine strategy**

I. INTRODUCTION

Production of vaccine and other bio-pharmaceuticals in plants is one of the major application of applied biotechnology (Malabadi, 2008; Malabadi *et al*. 2010, 2011, 2012e; Rybicki, 2010; Mason *et al*. 1992, 1996). Therefore, plants have a great potential as photosynthetic factories for lower cost production of biomedicines (Walmsley and Arntzen, 2003; Mason *et al*. 1992, 1996). The superiority of plant made vaccines also comes from their increased

immunity response as compared to traditional mammalian counterparts (Malabadi *et al*. 2012e; Ma *et al*. 1997, 1998 2004, 2005; Daniell *et al*. 2009; Davoodi-Semiromi *et al*. 2009, 2010; Walmsley and Arntzen, 2003; Rybicki, 2010). Autotrophic plants are also considered as the superior recombinant protein expression systems in terms of a high quality, increased quantity of desired protein which could be produced within a short period of time (Malabadi *et al*. 2012e; Davoodi-Semiromi *et al*. 2009, 2010; Mason *et al*. 1992, 1996). Further plant expression systems have no risk of product contamination by viruses of mammalian origin (Malabadi, 2008; Ma *et al*. 1997, 1998 2004, 2005; Daniell *et al*. 2009; Davoodi-Semiromi *et al*. 2009, 2010). The expenditure associated with traditional production of vaccine methods could be reduced by using plant made pharmaceuticals (Malabadi, 2008;Davoodi-Semiromi *et al*. 2009, 2010; Walmsley and Arntzen, 2003; Daniell *et al*. 2009; Mason *et al*. 1992, 1996). There are two different methods; stable genetic transformation and transient gene expression system used for the production of recombinant proteins in plants (Newell, 2000; Bhoo *et al*. 2011; Gleba *et al*. 2007). The regeneration of stable transgenic plants is vey time consuming, and ends up in the lower yield of protein (Newell, 2000; Bhoo *et al*. 2011; Gleba *et al*. 2007). Therefore, transient expression enhances the protein production level since the regeneration of stable transgenic plants is a very slow process (Newell, 2000; Bhoo *et al*. 2011; Gleba *et al*. 2007). On the other hand transient gene expression system is robust, and accumulates a very high amount of recombinant protein in plants. Recently plant gene viral expression vectors were used to amplify gene copy number using syringe agroinfilteration technique for transient expression in plants. Therefore, the combination use of *deconsturcted viral vectors* and *agroinfection* technique provide a new platform for the development of number of plant virus vector system for the over expression of a gene of interest in plants (Gleba *et al*.2005, 2007, 2014; Mortimer *et al.* 2015; Malabadi *et al*. 2016). Tobacco mosaic virus (TMV), potato virus X (PVX), cowpea mosaic virus (CPMV), and alfalfa mosaic virus (AIMV) have been utilized to develop modern plant viral expression vectors (Yusibov *et al*. 2006; Bhoo *et al*. 2011). Recently as an improvement in plant gene viral expression studies, deconstructed tobacco mosaic virus (TMV)-based system called magnICON (Icon Genetics Inc., Germany) was created (Gleba *et al*. 2004; Marillonnet *et al*. 2004; Gleba *et al*. 2007; Bhoo *et al*. 2011; Malabadi *et al*. 2016). The magnICON (Icon Genetics Inc., Germany) vector system is one of the successful promising platform for the production of biopharmaceuticals in plants (Gleba *et al*. 2005; Bhoo *et al*. 2011).

In general, there are 5 methods to control and eradicate the infectious diseases. First one is the development of vaccine approach using different expression platforms such as 1) mammalian expression, 2) plant expression and 3) Chicken egg yolk antibody production IgY expression (For example, Sunwoo *et al*. 1996, 2010; Gujral *et al.* 2015; Bade and Stegemann,1984; Akita and Nakai, 1992; Paul *et al*. 2007;; Sudjarwo *et al.* 2012; Han *et al*. 2012; Megha *et al*. 2014; Qiu *et al*. 2014; Halfmann *et al*. 2014; Olinger-Jr *et al*. 2012; Zhang *et al*. 2014; Ganguly *et al*. 2013a, 2013b, 2013c, 2013d, 2014, 2015). The second approach is the development of antiviral/ antibacterial/ antifungal drug approach (Warren *et al.* 2014; Oestereich *et al.* 2014; Halfmann *et al*. 2014; Geisbert *et al*. 2010; Halfmann *et al*. 2014; Wong and Kobinger, 2015). The third new approach is the application of nanotechnology for a nanomedicine (Malabadi *et al*. 2012a, 2012b, 2012c, 2012d; Khan *et al*. 2011, 2012). The fourth approach is DNA vaccine approach which plays an important role in combating infectious diseases. In this approach, DNA plasmids can be used to induce a protective (or therapeutic) immune response by delivering genes encoding vaccine antigens (Ferraro *et al*. 2011; Saade and Petrovsky, 2012; Tregoning and Kinnear, 2014). Finally fifth approach is the direct oral consumption of herbal-medicinal plants as guided by local traditional healers in the developing countries (Malabadi *et al*. 2005; Malabadi, 2005; Malabadi and Vijaya Kumar, 2005, 2007, 2008; Malabadi *et al*. 2007; Malabadi *et al*. 2010). A large number of medicinal plants are attributed with various pharmacological activities owing to a diversified class of phytochemicals (Gleiser *et al*. 2007, 2011; Gillij *et al*. 2008; Chalannavar *et al*. 2011, 2012, 2013a, 2013b, 2015a, 2015b; Narayanaswamy *et al.* 2013, 2014a, 2014b; Malabadi *et al.* 2007, 2010; Bhat *et al*. 2014). Both rural and urban population still depends on the traditional healers for health care (Malabadi *et al.* 2007, 2010; Bhat *et al*. 2014). Traditional healers are knowledgeable about the plants and their medicinal values which is passed from one generation to the other (Malabadi *et al.* 2007, 2010; Bhat *et al*. 2014). There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal plants for the treatment of various human diseases. Many useful drugs from plants have been discovered by following up ethnomedical uses (Gleiser *et al*. 2007, 2011; Gillij *et al*. 2008; Malabadi *et al.* 2007, 2010; Narayanaswamy *et al.* 2013, 2014a, 2014b). For example few medicinal plants such as insulin plant, *Costus speciosus* (Malabadi, 2002a, 2005; Malabadi *et al*. 2004, 2005a), *Catharanthus roseus* (Malabadi *et al*. 2009), *Clitoria ternatea* (Malabadi and Nataraja, 2001, 2002; Malabadi, 2002b; Malabadi *et al.* 2005b), and papaya (*Carica papaya* L.) **(**Subenthiran *et al*. 2013; Malabadi *et al*. 2011) have been tested to control various human diseases like dengue, chikungunya and other infectious human diseases. The use of papaya leaf juice significantly increased the platelet count among dengue infected patients (Subenthiran *et al*. 2013). In case of a plant derived vaccine approach, deadly virus is not handled but a recombinant protein expressed in plant is directly used as an antigen for vaccination.

This review paper highlights the recent developments made in plant derived vaccines, antiviral drugs particularly against Ebola virus disease, also discussed about pathogenicity of the Ebola virus, and factors related to Ebola outbreak in West Africa.

II. EBOLA VIRUS; OVERVIEW OF THE DISEASE

African endemic Ebola viruses (EBOVs) belong to the family *Filoviridae* cause severe viral hemorrhagic fever in humans and nonhuman primates (Das *et al*. 2007; Sanchez *et al.* 2007; Shahhosseini *et al*. 2007; Kilgore *et al*. 2015; Kaushik *et al.* 2016). Ebola virus was first detected in 1970 in Tandala, Democratic Republic of Congo (DRC) followed by first Ebola virus outbreak occurred near Ebola river in Democratic Republic of Congo and Sudan in 1976 which claimed more than 300 lives (Team Roa, 1978; Heymann *et al*. 1980; Alexander *et al.* 2015; Kilgore *et al*. 2015). Ebola viruses (EBOVs) are enveloped, negative single-stranded RNA viruses with a genome of 19 kb in size (Das *et al*. 2007; Shahhosseini *et al*. 2007;Reed and Mohamadzadeh, 2007; Saijo *et al*. 2006; Marzi *et al.* 2013). Ebola virus is in filamentous form with no definite shape measuring approximately 80nm in diameter (Bhoo *et al*. 2011). The Ebola virus genome encodes 7 viral proteins such as nucleoprotein (NP), matrix protein (VP24), glycoprotein (GP), polymerase cofactor (VP35), replication-transcription protein (VP30), matrix protein (VP40), and RNA dependent RNA polymerase(L) with an additional soluble glycoprotein (sGP) (Chandran *et al.* 2005; Das *et al*. 2007; Sanchez *et al.* 2007; Reed and Mohamadzadeh, 2007; Saijo *et al*. 2006; Marzi *et al.* 2013). Ebola virus disease is one of the major health concern throughout the world due to the lack of any approved medicine or vaccine for controlling the disease in humans (Sanchez *et al.* 2007;Geisbert *et al*. 2010; Saijo *et al*. 2006; Marzi *et al.* 2013). The US Centers for Disease Control identified both Ebola and Marburg viruses as "category A" bioterrorism agents (Bhoo *et al*. 2011). Generally human are infected with Ebola viruses through close contact with contaminated blood samples, tissues, or excretions viremic animals including patients with Ebola infections (Saijo *et al*. 2006; Shahhosseini *et al*. 2007; Sanchez *et al.* 2007; Marzi *et al.* 2013). Ebola infected patients develop a flu like symptoms followed by fever, chills, malaise and myalgia within a incubation period of 4 to 10 days (Saijo *et al*. 2006; Marzi *et al.* 2013; Falzarano *et al*. 2011). In a later stage, Ebola infected patients also suffered from anorexia, nausea, muscle pain, nose bleeding, vomiting blood, and a characteristic rash, vomiting, abdominal pain, diarrhea, headache, confusion and coma (Saijo *et al*. 2006; Das *et al*. 2007; Shahhosseini *et al*. 2007). In a later advanced stage of Ebola virus hemorrhagic fever disease, bleeding in liver, stool, spleen, kidney, and stomach is a serious and uncontrolled issue leading to the multiorgan failure coupled with shut down of immune system (Saijo *et al*. 2006; Kaushik *et al.* 2016). Many Ebola infected patients were not survived due to disseminated intravascular coagulopathy (Saijo *et al*. 2006). However, the pathogenesis of the Ebola disease is still poorly understood. Ebola virus (EBOV) consists of 5 species, Zaire EBOV, Bundibugyo EBOV, Sudan EBOV, Ivory Coast EBOV, and Reston EBOV which were first isolated from Democratic Republic of Congo, Uganda, Sudan, Ivory Coast and the Philippines, respectively (Saijo *et al*. 2006; Shahhosseini *et al*. 2007; Feldmann and Geisbert, 2011). Further the genomes of the five different Ebola viruses are different in sequence and the number and location of gene overlaps. Among different Ebola virus (EBOV) species, ZEBOV (Zaire Ebola virus) is a highly virulent pathogen, which kills 90% of the infected patients, due to haemorrhagic fever (Geisbert *et al*. 2010; Saijo *et al*. 2006; Marzi *et al.* 2013). The 2014 outbreak of Ebola haemorrhagic fever (EHF) in West African countries (Sierra Leone, Guinea, and Liberia) caused by ZEBOV (Zaire Ebola virus) is the largest outbreak of Ebola haemorrhagic fever (EHF) in history (Saijo *et al*. 2006; Marzi *et al*. 2013; Du Toit, 2014; Kaushik *et al.* 2016; Alexander *et al*. 2015; Pinzon *et al*. 2004; Frieden *et al*. 2014; Rivers *et al*. 2014).

Hunting and consumption of 'bushmeat' which is a critical source of protein is another cause of Ebola outbreak in Western Africa (Du Toit, 2014; Alexander *et al.* 2015). Fruit Bats were identified as the natural reservoirs of the Ebola viruses since fruit bats were capable of supporting uncontrolled Ebola virus replication without becoming ill (Nguyen *et al.* 2015). Fruit bats being the primary natural host for Ebola virus, were smoked, dried and roasted to a fine powder often mixed with spices, consumed with rice or corn paste or grilled meat as a protein source by people of West-Africa (Sierra Leone, Guinea, Liberia and Nigeria) (Leroy *et al*. 2005; Saeidnia and Abdollahi, 2014; Pourrut *et al*. 2005;Hoenen *et al*. 2012; Du Toit, 2014;Nguyen *et al.* 2015). However, transmission of Ebola viruses within bat populations remain unknown (Leroy *et al*. 2005; Du Toit, 2014;Nguyen *et al.* 2015). In addition to this, plants, arthopods and birds were also considered as the source of the Ebola viruses in Western Africa (Saeidnia and Abdollahi, 2014; Du Toit, 2014; Alexander *et al.* 2015). Furthermore, Ebola outbreaks are mostly originated from an individual who handles the carcass of gorilla, chimpanzee or Duiker (Saeidnia and Abdollahi, 2014; Pourrut *et al*. 2005; Swanepoel *et al*. 1996; Peterson *et al*. 2004). In another possibility, the Ebola virus might have transmitted from wildlife to people through contact with infected fruit bats, and through intermediate hosts, such as gorilla's, monkeys, apes, or pigs that have themselves become infected through contact with fruit bat saliva or faeces (Leroy *et al*. 2005; Na *et al*. 2015; Alexander *et al.* 2015; Kaushik *et al.* 2016). Ebola virus can spread from an infected person to others through direct contact with blood or body fluids such as saliva, sweat, feces, breast milk, and semen or objects viz, needles that have been contaminated with the virus and infected fruit bats or primates (Nguyen *et al*. 2015). The first evidence for the presence of Zaire Ebola virus in naturally infected fruit bats was documented by detection of viral RNA and antibodies in three tree-roosting bat species: *Hypsignathus monstrosus*, *Epomops franqueti*, and *Myonycteris torquata* (Leroy *et al*. 2005; Feldmann and Geisbert, 2011; Pourrut *et al.* 2007; Alexander *et al.* 2015).

Ebola hemorrhagic fever is still very commonly found disease in the Africa region (Alexander *et al*. 2015; Pinzon *et al*. 2004; Frieden *et al*. 2014; Rivers *et al*. 2014).

There are many factors which influenced the 2014 outbreak of Ebola virus in West Africa reviewed by Alexander *et al*. (2015). According to Alexander *et al.* (2015), the prominent factors are, 1) Lack of public awareness of the Ebola disease in remote villages 2) Stigmatization of Ebola virus disease outbreak 3) Hunting and consumption of bushmeat 4) Traditional burial practices 5) Spill over of Ebola virus from the wildlife reservoir to human populations 6) Chimpanzees unique behaviour leading to Ebola spill over pathways 7) Ability of migration of straw-coloured fruit bat (largest population of bats species in Africa) (Alexander *et al*. 2015) to long distances (up to 2,500km) leading to movement of Ebola from Central Africa to West Africa 8) Consumption of fruits by villagers that has already been contaminated with infected fruit bat saliva or feces 9) Behavioural and cultural practices 10) Socio-ecological conditions 11) human-mediated environmental change and 13) Human mobility from rural-to-urban migration (Alexander *et al*. 2015; Pinzon *et al*. 2004; Frieden *et al*. 2014; Rivers *et al*. 2014).

Now a days Ebola virus disease is one of the major public health threat in Africa since Ebola virus is very dangerous poses high individual risk of laboratory infections under hospital settings which is fatal resulting in the higher rate of infection (Na *et al*. 2015; Kaushik *et al.* 2016). Therefore, handling of live Ebola virus and related Ebola virus experiments have to be performed in biosafety level 4 (BSL-4) laboratories (Na *et al*. 2015; Kaushik *et al.* 2016). However, biosafety level 4 laboratories with certified infrastructure to handle Ebola infected patient specimen are only available in a very few countries such as India (National institute of Virology (NIV), Pune, Maharashtra state; High Security Animal Disease Laboratory (HSADL), Bhopal, Madya Pradesh state; Centre for Cellular and Molecular Biology (CCMB), Hyderabad, Telangana state; All India Institute of Medical Sciences (AIIMS), New Delhi, Defence Research Developmental Laboratories (DRDO), Bhopal, Madya Pradesh state), Congo (Institute National de Recherche Bio-Médicale Mobile lab in Democratic Republic of Congo), UK (National Institute for Medical Research, London; Public Health England Mobile lab. London), USA (Texas Biomedical Research Institute, San Antonio, Texas; The Galveston National Laboratory BSL-4 (P4) lab on the Campus of the University of Texas Medical Branch; NIAID Rocky Mountain Laboratories, Hamilton, Montana; United States Centers for Disease Control (CDC); US First Area Medical Laboratory; US National Institutes of Health, and US Naval Medical Research Center Mobile Lab), US Army Medical Research Institute of Infectious Diseases (USAMRIID), Canada (National Microbiology Laboratory, Winnipeg, Manitoba; Public Health Canada Mobile Lab), France (Jean Mérieux laboratory, Paris; Institute Pasteur Dakar; Institute Pasteur Lyon; Institute Pasteur Paris), Germany (Robert Koch Institute, Berlin; Bernhard Nocht Institute for Tropical Medicine, Hamburg; Friedrich Loeffler Institute on the Isle of Riems, Greifswald; Philipps University of Marburg, Marburg), China (Wuhan Institute of Virology of the Chinese Academy of Sciences, Hubei, Wuhan), Japan (National Institute for Infectious Diseases, Tokyo, Musashimurayama), South Africa (National Institute for Communicable Diseases, Johannesburg), Sweden (European Union Mobile Laboratory Consortium (EM Lab, Solna), and Russia (State Research Center of Virology and Biotechnology VECTOR, Novosibirsk, Oblast, Koltsovo; Russian Rospotrebnadzor Mobile Lab) (Na *et al*. 2015).

III. EBOLA VIRUS; PLANT MADE VACCINE PLATFORMS

Till today there is no medically approved therapeutic drug or vaccine in market against Ebola virus even after the disease was detected in 1976. Therefore, Ebola haemorrhagic fever remains a plague for the population of Western Africa, showing an increase in the number of death cases (Chandran *et al*. 2005; Feldmann and Geisbert, 2011; Du Toit, 2014; Swamy *et al*. 2014). Furthermore, Ebola virus basic research is very limited due to high biosafety classification of Ebola virus (level-4) (Nguyen *et al.* 2015). Many laboratories in developed countries are working on the development of the vaccine for Ebola since 2014 outbreak in West Africa is the wake up call of a major threat to human population (Halfmann *et al*. 2014). Very recently few experimental Ebola virus vaccines have been developed, and these vaccines showed varying degrees of immunity levels in animal models. Among the experimental Ebola virus vaccine platform, a replication-competent vesicular stomatit is virus (VSV) expressing Ebola virus glycoprotein(s) (the major viral immunogen) has shown very promising and effective immunity level titers against Ebola in nonhuman models (Marzi *et al*. 2011;Halfmann *et al*. 2014). In another development a replication-defective, chimpanzee adenovirus vector, ChAd3 have been developed as another experimental Ebola vaccine platform. During this study a single dose of 1010 recombinant adenovirus particles expressing the glycoproteins of Zaire ebolavirus conferred immunogenic against Zaire Ebola virus disease in most of the infected animals (Stanely et al, 2014; Halfmann *et al*. 2014). Because of this promising result, the chimpanzee adenovirus vector, ChAd3 has now entered a Phase I clinical trial to test its safety, tolerability, and immunogenicity in human volunteers (Halfmann *et al*. 2014). Very recently a particular cocktail of three monoclonal antibodies against Zaire EBOV virus (ZEBOV) glycoprotein called as ZMapp drug (developed jointly by Mapp Biopharmaceutical Inc., San Diego, California 92121, USA; Public Heatlth Agency of Canada, and Defyrus Inc, Toronto, Canada) has been used as an experimental Ebola vaccine platform (Qiu *et al*. 2014; Halfmann *et al*. 2014; Olinger-Jr *et al*. 2012; Zhang *et al*. 2014; Wong and Kobinger, 2015). The significance of ZMapp drug is that antibodies used in the ZMapp drug were produced in tobacco plant (*Nicotiana benthamiana*) manufactured by Kentucky Bio-Processing unit (Owensboro, KY, USA) under a contract from Mapp biopharmaceuticals Inc., (Owensboro, KY, USA)(Qiu *et al*. 2014; Halfmann *et al*. 2014; Olinger-Jr *et al*. 2012; Zhang *et al*. 2014; Choi *et al*.

2015). This particular cocktail of 3 monoclonal antibodies [This is an improved IgG MAb cocktail comprising MAbs from 2 precursor cocktails, ZMAb (providing MAbs, c2G4 and c4G7) and MB-003 (providing MAb, c13C6)] provided complete protection against ZAIRE EBOV (ZEBOV) virus in infected non human primates (Qiu *et al*. 2014; Halfmann *et al*. 2014; Zhang *et al*. 2014; Wong and Kobinger, 2015). Hence US Food and Drug Administration (FDA) approved tobacco plant made ZMapp antibody cocktails under the emergency compassionate use in patients infected with Ebola virus during the current outbreak (Halfmann *et al*. 2014; Choi *et al*. 2015; Wong and Kobinger, 2015). During this experimental immunization programme, seven of Ebola virus infected patients were treated with tobacco plant derived ZMapp antibody cocktail (Olinger-Jr *et al.* 2012; Halfmann *et al*. 2014; Wong and Kobinger, 2015). In this experiment, 5 patients survived their Ebola virus infection, and unfortunately at least two individuals have died from the Zaire. ebola virus infection despite receiving ZMapp antibody cocktail (Lyon *et al*. 2014; Qiu *et al*. 2014; Halfmann *et al*. 2014; Wong and Kobinger, 2015). Therefore, tobacco plant produced ZMapp antibody cocktails results are uncertain in humans and demands future clinical trials for the approval of medicine since current stocks of ZMapp have been exhausted (Qiu *et al*. 2014; Halfmann *et al*. 2014; Wong and Kobinger, 2015). This wave of plant derived antibody has opened a new ray of hope for the commercialization of plant made vaccine platform. The study conducted by Mapp biopharmaceutical Inc., Sandiago, USA, confirmed that antibodies expressed by magnifection technique using magnICON expression system (Icon Genetics Inc., Germany) (Gleba *et al*. 2005, 2007, 2014) in the glycomodified tobacco (*Nicothiana benthamiana*) plants had superior anti Ebola virus efficacy in animal models compared to other expression platforms (Qiu *et al*. 2014; Halfmann *et al*. 2014; Olinger-Jr *et al*. 2012; Zhang *et al*. 2014; Wong and Kobinger, 2015). In general wild type plants glycosylate proteins of interest but glycans carry residues of xylose and fucose in a non-mammalian linkage (Zhang *et al*. 2014). Therefore, transgenic *N. benthamiana* plants with with fucosyl- and xylosyl-transferase knocked down plants were generated. Antibodies produced in these glycomodified tobacco plants had mammalian-like glycans (Zhang *et al*. 2014). Phoolcharoen *et al.* (2011) reported the use of the compounds such as polyinosinic:polycytidylic acid (PIC, a Toll-like receptor 3 agonist) as highly effective adjuvant agent during mice immunization study. After vaccinating mice with tobacco plant (*N.benthamiana*) derived Ebola Immune Complexes (EIC) plus PIC, 80% of the animals were protected against a lethal challenge with live Ebola virus (Phoolcharoen *et al.* 2011). In another study, a geminiviral replicon system was used to produce an Ebola immune complex (EIC) in tobacco plants leaves (*Nicotiana benthamiana*) by using syringe agroinfilteration technique (Bhoo *et al*. 2011). Here Ebola glycoprotein (GP1) was fused at the C-terminus of the heavy chain of humanized 6D8 IgG monoclonal antibody, which specifically binds to a linear epitope on GP1(Bhoo *et al*. 2011). Co-expression of the GP1-heavy chain fusion and the 6D8 light chain using a geminiviral vector in leaves of *Nicotiana benthamiana* produced assembled immunoglobulin (Bhoo *et al*. 2011). Subcutaneous immunization of BALB/C mice with purified Ebola Immune Complex (EIC) resulted in anti-Ebola virus antibody production at levels comparable to those obtained with a GP1 virus-like particle (Bhoo *et al*. 2011). These results show excellent potential for a plant-expressed Ebola Immune Complex (EIC) as a human vaccine (Bhoo *et al*. 2011).

IV. EBOLA VIRUS; ANTIVIRAL DRUG PLATFORMS

The antiviral drugs such as the nucleoside analogs T-705 (favipiravir) and BCX4430 tested in rodent and nonhuman primate models also inhibited Ebola viral RNA synthesis (Warren *et al.* 2014; Oestereich *et al.* 2014; Halfmann *et al*. 2014). In addition to this, both compounds also inhibit RNA synthesis of other medically important human RNA viruses (Warren *et al.* 2014; Oestereich *et al.* 2014; Halfmann *et al*. 2014). These experimental results suggests that T-705 is a candidate for treatment of Ebola virus disease (Oestereich *et al.* 2014). Hence, the pyrazinecarboxamide derivative T-705 (favipiravir) is now approved in Japan to control pandemic influenza (Halfmann *et al*. 2014). In another study, lipid nanoparticles/small interfering RNA technology have also entered clinical trials which plays an important role to combat Ebola virus infections in nonhuman primates (Geisbert *et al*. 2010; Halfmann *et al*. 2014). Brincidofovir (BCV)(developed by Chimerix, USA) is a lipid-conjugated analog of cidofovir, is another FDA approved drug which showed antiviral activity against Ebola viruses in animal models (Wong and Kobinger, 2015; Kilgore *et al*. 2015). Several patients infected with Ebola virus were survived by using this drug, except few were unable to survive despite using drug Brincidofovir (BCV) (Wong and Kobinger, 2015; Kilgore *et al*. 2015). In another recent development reported by Dr.Travis Warren and co-workers at the US Army Medical Research Institute of Infectious Diseases (USAMRIID), a nucleotide prodrug GS-5734 inhibited Ebola virus RNA replication process and provided full protection to monkeys when treated 3 days after the deadly infection (Warren *et al*. 2015). Lamivudine (developed by GlaxoSmithKline, United Kingdom), a nucleoside analog of cytidine, is a reverse transcriptase inhibitor (Wong and Kobinger, 2015). During this West African 2014 outbreak, a Liberian doctor used lamivudine to treat 15 infected patients, with 13 eventually surviving EBOV disease (Wong and Kobinger, 2015). TKM-Ebola (developed by Tekmira Pharmaceuticals, Canada) consists of a cocktail of three siRNAs in the form of lipid nanoparticles, designed specifically to target regions in three EBOV genes: EBOV membrane-associated protein 24 (VP24), the EBOV polymerase complex protein VP35, and polymerase (L) (Wong and Kobinger, 2015). A clinical trial of this drug is still under way and results are still awaited

(Wong and Kobinger, 2015). Another FDA approved drug candidate used to control Ebola virus infection is toremifene (Wong and Kobinger, 2015). The activity of toremifene was also evaluated by using a mouse model of EBOV infection (Wong and Kobinger, 2015). These experiments concluded that the infected animals were protected and survived following successful toremifene drug vaccination (Wong and Kobinger, 2015).

V. CONCLUSION

Ebola hemorrhagic fever is one of the viral pathogenic deadly disease, and till today there is no medical treatment or a approved vaccine for human use in market. The West African Ebola virus disease outbreak in 2014 is global issue of major threat to human population which has urged scientific community to develop a vaccine for Ebola virus disease. The experimental tobacco plant made ZMapp antibody cocktails (developed jointly by Mapp Biopharmaceutical Inc., San Diego, California 92121, USA; Public Health Agency of Canada, and Defyrus Inc, Toronto, Canada) tested against Zaire Ebola (ZEBOV) virus has opened up a new ray of hope for the commercialization of plant derived vaccines. Plant derived biopharmaceuticals provide promising platform but face many challenges before commercialization. The first challenge is how to get funding for the Ebola virus vaccine projects, then regulatory issues, and how to convince public about transgenic tobacco plant derived vaccine. Finally most of our study related to plant made vaccine is an experimental proof of concept in the laboratory settings since the last 26 years, and needs to be commercialized. Few of the plant made vaccines entered into clinical trials but not yet reached commercial market. The new glycosylation pathways could engineer plants for humanization of vaccines. The use of plant virus expression systems coupled with agroinfection technique can produce a large amount of recombinant protein within a short period of time. Therefore, MagnICON' vectors (Icon Genetics Inc., Germany) in their transient formats are widely used to produce higher levels of recombinant protein in plants since this system is powerful and easy to adopt in tobacco plants. Therefore, photosynthetic manufacturing is safe and worth for commercialization in a near future for controlling deadly diseases.

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